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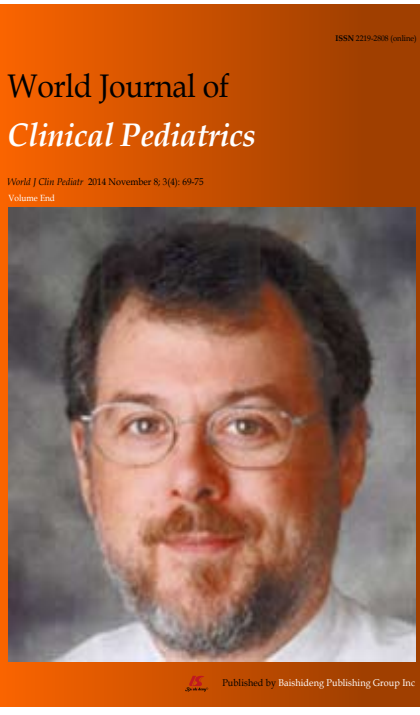

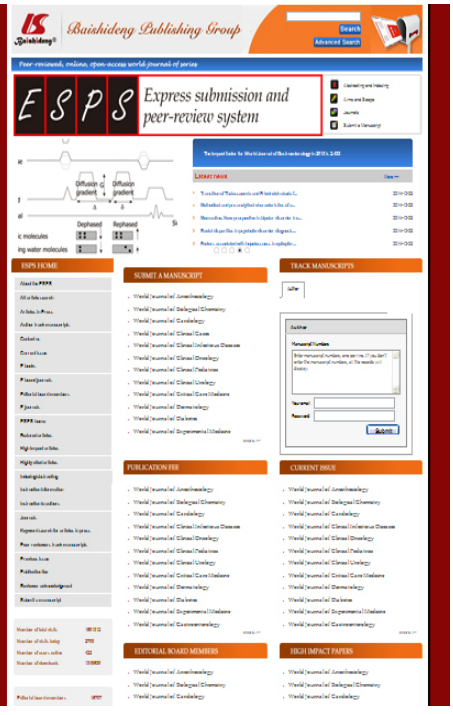

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Recent management of urinary stone disease in a pediatric population

Ozgu Aydogdu, Ayhan Karakose, Orcun Celik, Yusuf Ziya Atesci

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Abstract

The incidence of stone disease has been increasing and the risk of recurrent stone formation is high in a pediatric population. It is crucial to use the most effective method with the primary goal of complete stone removal to prevent recurrence from residual fragments. While extracorporeal shock wave lithotripsy (ESWL) is still considered first line therapy in many clinics for urinary tract stones in children, endoscopic techniques are widely preferred due to miniaturization of instruments and evolution of surgical techniques. The standard procedures to treat urinary stone disease in children are the same as those used in an adult population. These include ESWL, ureterorenoscopy, percutaneous nephrolithotomy (standard PCNL or mini-perc), laparoscopic and open surgery. ESWL is currently the procedure of choice for treating most upper urinary tract calculi in a pediatric population. In recent years, endourological management of pediatric urinary stone disease is preferred in many centers with increasing experience in endourological techniques and decreasing sizes of surgical equipment. The management of pediatric stone disease has evolved with improvements in the technique and a decrease in the size of surgical instruments. Recently, endoscopic methods have been safely and effectively used in children with minor complications. In this review, we aim to summarize the recent management of urolithiasis in children.

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Key words: Stone disease; Pediatric population; Urolithiasis; Surgical management

Core tip: The incidence of stone disease has been increasing and the risk of recurrent stone formation is high in a pediatric population. In recent years, endourological management of pediatric urinary stone disease is preferred in many centers with increasing experience in endourological techniques and decreasing sizes of surgical equipment. The management of pediatric stone disease has evolved with improvements in the technique and a decrease in the size of surgical instruments. Recently, endoscopic methods have been safely and effectively used in children with minor complications. In this review, we aim to summarize the recent management of urolithiasis in children.

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INTRODUCTION

Children with urinary stone disease represent the high risk group for stone recurrence[1]. Since the recurrence rate is higher in children compared to adults, ideally no residual stone fragments should be left behind after any treatment for urinary stones. A previous study showed that 69% of children with residual stone fragments ≤

5 mm following extracorporeal shock wave lithotripsy (ESWL) had an increase in stone size[2].

The standard procedures to treat urinary stone disease in children are the same as those used in an adult population. These include ESWL, ureterorenoscopy (URS), percutaneous nephrolithotomy (standard PCNL or mini-perl), laparoscopic and open surgery. ESWL is currently the procedure of choice for treating most upper urinary tract calculi in a pediatric population[3]. In recent years, endourological management of pediatric urinary stone disease is preferred in many centers with increasing experience in endourological techniques and decreasing sizes of surgical equipment[1,3]. This review aims to summarize the recent management of children with urinary stone disease.

ESWL

The use of ESWL in the treatment of urinary stones in a pediatric population was first reported by Newman *et al*[4] in 1986. ESWL is the preferred treatment in pediatric urinary stone patients with uncomplicated upper urinary tract calculi $\leqslant$ 15 mm[1,3,5,6]. Although stone free rates after ESWL in children range between 68% and 92%, recent stone free rates are difficult to interpret from the current literature due to discrepancies among trials with regard to the lithotripter model used, number of shocks administered and re-treatment rates[1,3,7,8]. Factors which decrease ESWL success rates include increased mean stone burden, increased infundibular length, infundibulopelvic angle greater than 45 degrees, harder stones such as cysteine and whewellite, and lower pole localization[1,9]. In a recent study, the authors aimed to define the preoperative kidney and stone characteristics on noncontrast-enhanced computed tomography that affect the success of ESWL for treatment of renal calculi in pediatric patients. The authors concluded that stone attenuation $\leqslant$ 600 HU and stone length $\leqslant$ 12 mm were significant independent predictors of ESWL success in children[10]. ESWL monotherapy has superior success rates in children compared to adults due to relatively softer stone composition, smaller relative stone volume, smaller body volume to facilitate shock transmission, and easier spontaneous stone passage due to increased ureteral compliance to accommodate stone fragments[1,3,11-13]. In children, ureteral stenting before ESWL is not needed as often as in adults and it is not clear if ureteral stent placement improves stone free outcomes[1,3,11]. ESWL can cause minor complications, including hematuria, perirenal hematoma, bruising and renal colic[1]. Although ESWL has low complication rates, the stone free rate after a single session is approximately 45%[3,14]. The need for multiple ESWL sessions is controversial since anesthesia is required and the effects of shock waves on renal tissue are not clear[1,3,15].

In a previous study, the authors found no negative effect of ESWL on renal function or blood pressure[16]. In another study, the authors found no significant changes in blood pressure or signs of acquired parenchymal renal scarring following ESWL in children[17]. Vlajković *et al*[18] evaluated glomerular filtration rate (GFR) before and after ESWL. The authors showed that GFR normalized or improved at the 3rd month after the ESWL procedure and concluded that ESWL is a safe treatment in children. In a previous epidemiological and questionnaire based retrospective study, the authors found that the patients treated with ESWL had an increased risk of developing hypertension and diabetes mellitus when compared to controls[19]. In contrast, in a different study, the authors prospectively examined 12 patients after ESWL and concluded that it is unlikely that ESWL and diabetes mellitus are related[20].

PCNL

After it was first described in children in 1985, several studies reported the use of PCNL in children with urinary stone disease using adult sized surgical instruments with high success and acceptable complication rates[21-24]. In 1998 Jackman *et al*[25] introduced a novel percutaneous access technique (mini-perc) and reported a 85% total success rate. The authors listed the benefits of this new technique as increased maneuverability, decreased blood loss and shorter hospital stay, with limitations including prolonged operative times and potential impairment of visualization during the procedure, especially for larger stones.

In a previous trial, the authors compared the results of percutaneous nephrolithotomy and shock wave lithotripsy for the treatment of 1 to 2 cm renal stones in children. They concluded that percutaneous nephrolithotomy is better than shock wave lithotripsy for treatment of 1 to 2 cm renal stones in children, yielding higher stone free and lower re-treatment rates[26].

Recently, the success rate of PCNL in a pediatric population was reported to be between 87% and 98.5%[11,23,26,27]. In a previous study, the authors reported the outcomes of 56 children who underwent PCNL[11]. The authors found a stone free rate of 89.8% and that the number and size of the access tracts were significantly associated with a postoperative hemoglobin decrease and transfusion rate. In a different study, the authors reported a 87% stone free rate following a PCNL procedure in 52 children with a mean age of 7.9 years[27]. The authors reported postoperative fever in 30% of the patients included in the study and a blood transfusion rate of 24%.

The European Association of Urology guidelines state that ESWL is the first choice for treating most renal pediatric stones and PCNL can be preferred for larger and complex stones. The guidelines also mention that PCNL can be used as monotherapy in most cases but is also used as an adjunctive procedure to other therapies[28]. Relative indications for PCNL as a primary therapy in a pediatric population include upper pole stones $\geqslant$ 1.5 cm, lower pole stones $\geqslant$ 1 cm, harder stones and potential anatomical abnormalities that can possibly impair urinary

drainage and thus stone clearance, such as ureteropelvic junction obstruction and ureter stricture[1,3]. Potential limitations for the use of a PCNL procedure in children include possible parenchymal damage and associated impairment in renal function, radiation exposure and the risk of major complications, including urinary sepsis and bleeding[3]. Some studies evaluated the potential loss of renal function due to renal scarring after PCNL in children[29,30]. In a previous study, the authors investigated renal scarring on a dimercaptosuccinate (DMSA) renal scan following PCNL and found no renal scarring on DMSA[29]. In the same study, a diethylenetriamine pentaacetic acid renal scan was used to follow-up renal function after PCNL and it was observed that renal function had improved or not changed, except for one patient.

PCNL can be used both as monotherapy and in combination with ESWL in children[3,7]. The use of PCNL in combination with ESWL is preferred to reduce the number of access tracts and associated morbidity rates. In a previous study, the authors reported a 60% stone free rate after PCNL. The stone free rate increased to 100% following an ESWL procedure[30]. In a similar study, the authors reported a 59% monotherapy stone free rate after PCNL in 169 children[24]. Thirty-four point five percent of children with residual stones were treated with ESWL and the overall stone free rate increased to 93.8%. Although PCNL is an invasive treatment, in experienced hands it can be effectively and safely used in children with large stone burdens with the use of smaller sized surgical instruments and more efficient energy sources used for stone fragmentation.

URS

Although previously URS was only used for ureter stones below the iliac crest and for upper urinary tract stones following an unsuccessful ESWL procedure in children, many clinicians prefer to use URS even in young children with the introduction of smaller diameter ureteroscopic instruments and holmium laser[1,3]. URS was first used in 1988 for distal ureteral calculi in children and the authors reported stone free rates between 86%-100% in the early series[1,7,12,13]. In a previous randomized study, the authors compared URS and ESWL in 31 children and found stone free rates of 94% and 43% in the URS and ESWL groups respectively[31]. In a different study, the authors reported their experience using 4.5, 6 and 8 Fr rigid URS for treating proximal ureteral stones in 24 children and reported a stone free rate of 100%[32]. Corcoran *et al*[33] reported the outcomes of 47 children with upper tract stones treated with flexible URS and holmium laser lithotripsy. They reported a stone free rate of 88% and 26% in the children requiring staged procedures.

There was a concern regarding the use of URS in children with urinary tract stones due to potential complications, including ureteral ischemia, ureteral stricture and vesicoureteral reflux (VUR). However, a previous review of 221 URS procedures in a pediatric population showed that only two children had ureteral strictures and eight had low grade VUR[13]. The introduction of flexible ureteroscopes which can bend up to 270° made the removal of renal stones in lower calices possible[1]. In a previous study, the authors reported the success rate of lower pole calculi removal as 76% in 21 children with a mean age of 15 years[34]. In a prospective randomized study comparing ESWL and URS for lower pole calculi up to 1 cm, after three months, 35% and 50% of the patients in the ESWL and URS groups respectively were stone free[35].

Smaller and more durable endoscopes with the introduction of laser technology for the fragmentation of urinary stones allow the use of URS in children to be more prominent. Relative contraindications for URS in children include staghorn stones, anatomical anomalies and previous unsuccessful endoscopic procedures[3].

LAPAROSCOPIC/ROBOTIC/OPEN NEPHROLITHOTOMY

Surgical treatment of children with larger stones is technically challenging. Open surgery is used more in developing countries compared to developed countries, probably due to cost effectiveness[1,3]. Open surgery is preferred in children with concomitant anatomical abnormalities, including ureteropelvic junction obstruction and obstructive megaureter[1,36]. In a previous study, the authors reported a 95.4% stone free rate in children who underwent open nephrolithotomy[37]. In a different study, the authors reported a success rate of 100% with no major complications in 8 children who underwent laparoscopic pyelolithotomy[38]. Lee *et al*[39] reported the outcomes of 5 children who were treated with robotic-assisted laparoscopic pyelolithotomy and mentioned that the technique is feasible and safe as an alternative to open surgery in children.

CONCLUSION

The management of pediatric stone disease has evolved with improvements in the techniques and a decrease in the size of surgical instruments. Recently, endoscopic methods have been safely and effectively used in children with minor complications.

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BRIEF ARTICLE

Evaluation of oxidant-antioxidant status in overweight and morbidly obese Saudi children

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Abstract

AIM: To evaluate the antioxidant enzymes and oxidative products in overweight and obese Saudi children before the onset of metabolic complications.

METHODS: The study was carried out on 231 Saudi children. They were classified into three groups: uncomplicated overweight, uncomplicated morbid obesity, and the matched age group as control. All subjects underwent anthropometric measurements and activities of superoxide dismutase, catalase, glutathione peroxidase (GSH-Px), glutathione reductase, the concentrations of reduced GSH, malondialdehyde (MDA) oxidized low-density lipoprotein (ox-LDL) and advanced oxidation protein products (AOPPs) were measured in the blood of these groups.

RESULTS: Overweight and obese children had a significantly higher body mass index, while obese children only had a significantly higher waist-to-hip ratio compared to that of the control group. The enzyme activities under study were significantly elevated in the overweight group, although they were significantly reduced among obese children. The concentration of GSH was reduced in both the overweight and obese groups. The mean values of ox-LDL, MDA and AOPP were non-significantly increased in overweight children, while they were significantly elevated in obese children compared to that of normal weight children. A significant disturbance of oxidant-antioxidant status was observed in severely morbid children.

CONCLUSION: The increase of oxidative stress in obese children is associated with the increase in AOPPs and MDA which reflects an imbalance between reactive oxygen species production and antioxidant defense.

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Key words: Saudi children; Body mass index; Malondialdehyde; Superoxide dismutase; Catalase; Glutathione peroxidase; Glutathione reductase; Reduced glutathione

Core tip: Childhood obesity is growing at an alarming rate and is concomitant with an increasing prevalence of oxidative stress. The association between obesity and oxidative stress is illustrated in the present study that showed that obese children with body mass index greater than 35 kg/m^2 had higher oxidative products, *e.g.,* malondialdehyde, advanced oxidation protein products and oxidized low-density lipoprotein concentrations with lower antioxidants, *e.g.,* superoxide dismutase, catalase, glutathione peroxidase (GSH-Px) and glutathione reductase, and GSH. Therefore, the early recognition of these changes in oxidant status in children is important for preventing the long-term complications of obesity and targeting individual subjects who are particularly at risk. In addition, improving the oxidant status in overweight and obese children may reduce obesity-related comorbidities in adulthood.

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INTRODUCTION

Obesity is one of the most common health problems among children and adolescents in developed and high resource countries[1]. In addition, it is a principal causative factor in the development of various diseases, such as dyslipidemia, atherosclerosis, cardiovascular and others, and increases the risk of premature illness and death later in life, raising public health concerns[2-4].

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet}$) and hydroxyl (·OH) radicals, with antioxidants defenses, which leads to oxidative damage of lipids, proteins and DNA and might be a major mechanism underlying obesity-related complications[5].

The human body has developed several mechanisms to protect biomolecules from the deleterious effects of ROS. These include the antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSG-R) and glutathione peroxidase (GSH-Px), as well as water and lipid-soluble antioxidants, such as GSH, ascorbate (vitamin C), α-tocopherol (vitamin E) and β-carotene[6]. They either detoxify ROS, convert superoxide radicals ($O_2^{\bullet}$) into H_2O_2, or metabolize peroxide organic molecules.

Glutathione protects the body organs against oxidative stress of ROS, either directly as an antioxidant or indirectly by maintaining other cellular antioxidants in a functional state[7].

The accumulation of oxidative modified macromolecules has been demonstrated in obese adults. The whole body oxidative stress is best reflected by systemic levels of lipid peroxidation, *e.g.,* malondialdehyde (MDA) and oxidized low-density lipoprotein (ox-LDL), which are considered the most reliable oxidative biomarkers[8]. The levels of MDA were significantly increased in obese children compared with non-obese children. ox-LDL, another marker of oxidative stress, is associated with obesity[9].

Proteins are recognized as oxidants by ROS, which then may undergo structural and functional modifications, leading to endothelial dysfunction. Advanced oxidation protein products (AOPPs) are novel markers of increased oxidative stress, which has some advantages over other markers because of their relatively early formation, greater stability, ease of determination and reliability, and also their longer life span[10], and are considered reliable markers to estimate the degree of oxidant-mediated protein damage.

Although the prevalence of morbid obesity and obesity-related complications in children has greatly increased in the eastern region of Saudi Arabia, the antioxidant enzymes and the more important oxidatively modified macromolecules, *e.g.,* MDA, ox-LDL and AOPP, have not been completely explored in overweight and obese children because only data on antioxidant vitamin levels are available[11-13]. The current study aims to evaluate the activities of SOD, CAT, GSH-Px, GSSG-R, levels of MDA, ox-LDL and AOPP in Saudi overweight, obese and normal weight children to identify obese children who are at risk of complications.

MATERIALS AND METHODS

Children and study protocol

The investigations were carried out in overweight and obese children referred to the Hospital Paediatric Clinic and attendees of the Polyclinic Center at King Faisal University in Al-Ahsa, Saudi Arabia between January 2011 and March 2012. The present study comprised a total of 213 children (6-12 years, mean age 9.5 ± 1.5 years; boys n = 143, 67% and girls n = 70, 33%). The children were free from endocrinological and liver disorders and genetic syndromes associated with obesity. Clinically they were stable without symptoms of any acute infections in order to avoid the possible influence of such conditions on the parameters examined. None of the children were smokers.

Children were classified into three groups: overweight, obese and healthy weight based on body mass index (BMI) (kg/m^2) using the International Obesity Task Force criteria[14]. Group I included 66 uncomplicated overweight children with a BMI = 25-30 kg/m^2 or 85th percentile < BMI < 95th percentile. Group Ⅱ included 83 children with uncomplicated morbid obesity of BMI ⩾ 95th percentile or BMI ⩾ 30 kg/m^2 or more. Group Ⅲ included 64 children of the same age as normal control of BMI = 18.5-25 kg/m^2 or < 85th percentile. BMI was calculated as weight in kilograms divided by height in meters squared[14]. BMI z-scores were calculated based on the United States Center for Disease Control and Prevention reference data[15].

Ethical approval

The study was approved by the Ethical Committee of the King Faisal University.

Data collection

Anthropometry: Anthropometric measures followed the protocols of the International Society for the Advancement of Kinanthropometry[16]. Height was measured with a wall-mounted stadiometer (SECA 770, Hamburg, Germany) in a relaxed position with arms hanging freely and without shoes to the nearest 0.3 cm. Weight was measured using electronic digital scales (TANITA ultimate scale 2000 scales, Tanita Corporation, Tokyo, Japan) to the nearest 0.1 kg, with children wearing only a hospital gown and underwear. Measurements were taken by a single technician to overcome inter-rater error.

Calculation of waist-to-hip ratio: To calculate waist-to-hip ratio (WHR), the waist circumference was measured at its smallest point between the iliac crest and the rib cage and the hip circumference at its largest width over the greater trochanters. Blood pressure was measured using a mercury gravity manometer with proper cuff size in standard conditions and ambulatory blood pressure monitoring was carried out[17].

Demographics/background information: Parents completed a questionnaire to collect information about household income, maternal education, child medications and any medical conditions and oral consent was given by all children.

Blood sampling: Blood samples were freshly withdrawn from the vein of various children groups after an overnight fasting on heparin as inpatients at the Hospital Pediatric Clinic and Polyclinic Center and were immediately transferred to our laboratory at the College of Medicine, King Faisal University in an icebox. Blood samples were immediately centrifuged at 4000 rpm at 4 ℃ and plasma was stored at -20 ℃ until analysis. The 50 μL of RBC were taken and lysed with 1.0 mL ice cold water and the clear lysate obtained after spinning down the cell debris at 8500 g for 10 min at 4 ℃ was used for the assays.

Biochemical analysis

Determination of hemoglobin (%): Determination of hemoglobin (Hb) was estimated spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) by using a kit obtained from Biodiagnostic Cairo, Egypt according to the method of Ranganathanand and Gunasekaran[18]. The values were expressed as g/dL.

Estimation of blood glucose: Blood glucose concentration was estimated spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) through application of the method described by Freund *et al*[19] using an enzymatic test kit (glucose oxidase) supplied by Biodiagnostic, Cairo, Egypt. The results were expressed as mg/dL.

Estimation of total serum protein: The total plasma proteins were estimated by using a spectrophotometric (Boeco S-20 Spectrophotometer, Hamburg, Germany) method of Buiret[20]. The results were expressed as g/dL.

Measurement of concentrations of oxidative products and activities of antioxidant enzymes: (1) Determination of MDA: MDA level, an end product of lipid peroxidation of erythrocytes, was assayed spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) using a diagnostic kit supplied by Biodiagnostic, Cairo, Egypt using the method of Stocks *et al*[21]. The results were expressed as nmol/g Hb; (2) Determination of plasma ox-LDL: ox-LDL level was estimated by using an enzyme-linked immunosorbent assay (Merocdia, Inc., Winston-Salem, NC, United States) kit according to the method described by Hoogerbrugge *et al*[22]. The concentration of ox-LDL was expressed in mg/g protein; and (3) AOPP: determination of AOPP was based on spectrophotometric detection of chloramin T at 340 nm according to the method of Witko-Sarsat *et al*[23]. Concentration of AOPP was expressed in chloramine units (μmol/g protein).

SOD activity (EC 1.15.1.1): The Jaiswal *et al*[24] method was used to estimate the total SOD activity spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) in RBC hemolysate by using a test kit obtained from SpinReact Biodiagnostic, Cairo, Egypt. The results were expressed as U/g Hb.

GSH-Px (EC 1.11.1.9): The activity of GSH-Px in erythrocytes was estimated spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) using the method described by Paglia *et al*[25] using a diagnostic kit provided by Biodiagnostic, Cairo, Egypt. The results were expressed as mU/g Hb.

GSSG-R (1.6.4.2): Erythrocyte GSSG-R activity was determined spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) using a diagnostic kit provided by Biodiagnostic, Cairo, Egypt, as described by Worthington *et al*[26]. The results were expressed as mU/g Hb.

CAT (EC 1.11.1.6): CAT activity was measured spectrophotometrically (Boeco S-20 Spectrophotometer, Hamburg, Germany) using a standard CAT assay kit Biodiagnostic, Cairo, Egypt, through following the decomposition rate of H_2O_2 at 240 nm, according to the method of Al-Essa *et al*[27]. The results were expressed as U/g Hb.

GSH: GSH was assayed using the method of Giustarini *et al*[28]. The results were expressed as mg/g Hb.

Statistical analysis

The data were reported as mean ± SD and analyzed with the SPSS 16.0.7 (SPSS, Chicago, IL, United States) for Microsoft Windows XP (Redmond, WA, United States) statistical software package. Differences between the groups were evaluated using the Student's independent samples *t* test. Group comparison was performed by using a one-way analysis of variance. Differences were considered statistically significant at $P < 0.05$. The graphic was drawn by Graph Pad Prism-5.

RESULTS

Basic characteristics

Clinical characteristics of the normal weight, overweight and obese children groups and their control, glucose and Hb are demonstrated in Table 1. A total of 213 children (normal weight = 66, overweight = 83, obese = 64) were included, with age range from 6-12 years (mean age = 9.7 ± 1.5); boys, *n* = 143; 67.1% and girls, *n* = 70; 32.9%. There were no differences in age, gender distribution and levels of fasting glucose and Hb between the different groups. Most of the overweight and obese children resided in the urban region. Body weight and BMI were significantly of higher levels among overweight and obese children compared to the controls. WHR were significantly higher in obese children than in the normal controls, whereas no difference was observed in the

Table 1 Characteristics, glucose and hemoglobin levels of obese and control children enrolled in the study *n* (%)

Characteristic	Subjects		
	Normal weight (*n* = 66)	Overweight (*n* = 83)	Obese (*n* = 64)
Gender			
Boys	42 (63.6)	60 (72.3)	41 (64.1)
Girls	24 (33.4)	23 (27.7)	23 (35.9)
Age, yr			
Mean ± SE	9.5 ± 1.2	9.7 ± 1.8	9.4 ± 1.5
P value	0.089	0.143[1]	0.163[1]
Residence			
Urban	44 (66.7)	55 (66.3)	40 (62.5)
Rural	22 (33.3)	28 (33.7)	24 (37.5)
Anthropometry			
Height in cm	141.9 ± 2.1	142.3 ± 2.4	141.1 ± 2.9
Mean ± SE			
P value		0.703[1]	0.994[1]
Body weight in kilogram		67.7 ± 5.4	139.7 ± 5.9
Mean ± SE	39.2 ± 3.4	0.0001[2]	0.0001[2]
P value		22.4 ± 2.3	32 ± 3.8
BMI		0.0001[2]	0.0001[2]
Mean ± SE	16.4 ± 1.7		
P value		0.89 ± 1.28	2.16 ± 2.64
BMI z-score		0.0001[2]	0.0001[2]
Mean ± SE			
P value	0.03 ± 0.32		
Waist/hip ratio			
Mean ± SE	0.77 ± 0.15	0.79 ± 0.16	0.88 ± 0.16
P value		0.602[1]	0.0004[2]
Age in years			
Mean ± SE	9.9 ± 1.6	9.9 ± 1.9	9.6 ± 2.1
P value		0.616[1]	0.724[1]
Glucose (mg/dL)			
Mean ± SE	80.1 ± 2.9	81.7 ± 2.3	83.5 ± 2.2
P value		0.719[1]	0.075[1]
Hb (g %)			
Mean ± SE	14.2 ± 1.1	13.9 ± 3.4	13.8 ± 3.9
P value		0.674[1]	0.741[1]
Systolic blood pressure (mmHg)			
Mean ± SE	115.6 ± 6.8	115.5 ± 7.4	116.2 ± 9.1
P value		0.874[1]	0.794[1]
Diastolic blood pressure (mmHg)			
Mean ± SE	68.8 ± 6.4	67.6 ± 5.1	68.8 ± 7.1
P value		0.728[1]	0.835[1]

Values are presented in means ± SE. BMI: Weight in kg/height in m^2. [1]non-significant values of overweight and obese groups *vs* control; [2]significant values of overweight and obese groups *vs* control. Comparison of the means was evaluated by ANOVA. BMI: Body mass index; Hb: Hemoglobin; ANOVA: Analysis of variance.

WHR of overweight children (Table 1).

Oxidant markers

Table 2 depicts the activities of antioxidant enzymes SOD, CAT, GSH-Px and GSSG-R in erythrocytes under study. The erythrocyte activities of SOD, CAT, GSH-Px and GSSG-R were significantly elevated in overweight children compared to the corresponding activities of the control group ($P < 0.001$). The mean activity values of these enzymes were decreased in the obese group compared to the corresponding activities of the normal weight and overweight groups ($P < 0.001$). The glutathione levels were decreased in both overweight and obese children in comparison to the corresponding level in the normal control group.

Table 2 Erythrocyte activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and reduced glutathione concentration

Group	SOD (U/g Hb)	CAT (mg/g Hb)	GSH-Px (mg/g Hb)	GSSG-R (mg/g Hb)	GSH (mg/g Hb)
Normal weight					
n	66	66	66	66	66
Mean ± SE	224.6 ± 5.3	85.4 ± 2.7	41.4 ± 2.6	42.4 ± 1.6	69.5 ± 1.9
Over weight					
n	83	83	83	83	83
Mean ± SE	252.7 ± 6.5	99.7 ± 3.7	56.8 ± 3.5	56.3 ± 2.5	66.7 ± 2.8
P value	0.001[1]	0.001[1]	0.001[1]	0.001[1]	0.01[1]
Obese					
n	64	64	64	64	64
Mean ± SE	181.7 ± 7.2	68.3 ± 5.1	37.3 ± 5.9	32.9 ± 4.6	33.3 ± 2.1
P value	0.001[1,2]	0.001[1,2]	0.001[1,2]	0.001[1,2]	0.001[1,2]

Values are presented in means ± SE. [1]Significant values of overweight and obese *vs* control; [2]Significant values of obese *vs* overweight group. Comparison of the means was evaluated by ANOVA. SOD: Superoxide dismutase; CAT: Catalase; GSH-Px: Glutathione peroxidase; GSSG-R: Glutathione reductase; GSH: Reduced glutathione concentration.

The concentrations of MDA, OxLDL and AOPP are shown in Figure 1 respectively. MDA, OxLDL and AOPP were non-significantly increased in the overweight children, although highly increased in obese children compared to the normal weight children ($P < 0.001$).

DISCUSSION

The present study estimated the glucose level to exclude the children with hyperglycemia because this condition may affect the levels of the present parameters. In addition, the present study avoided inherited obesity to exclude its effect on the same parameters and thus investigated the effect of the acquired fatness on the antioxidant status in children. The prevalence of overweight and obese children in the urban region may be attributed to habits of eating fatty foods and lack of physical activity due to the very hot weather.

Growing evidence indicates that the mitochondria of white adipose tissue (WAT), particularly of obese persons, are the main site of ROS generation, *e.g.,* superoxide radicals ($O_2^{\bullet-}$) and H_2O_2, accompanied by augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes[29]. This finding is confirmed by Mahadev *et al*[30] who reported that mRNA expression of NADPH oxidase increased in WAT of obese mice.

The antioxidant defenses against ROS include non-enzymatic, *e.g.,* thiol-containing compounds, and antioxidant enzymes[7]. The major antioxidant enzymes include SOD, GSH-Px, GSSG-R and CAT. In the present study, the overweight children showed an elevation in the activities of SOD, GSH-Px, GSSG-R and CAT, but their activities are depleted in obese children (Table 2). The activation of these antioxidant enzymes in overweight children may be to counteract the effect of oxidative

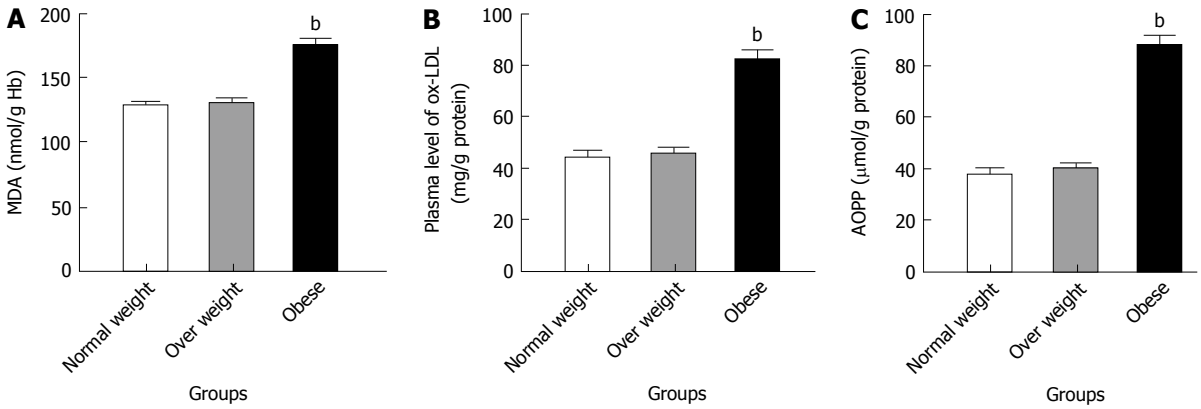

Figure 1 Erythrocyte levels of malondialdehyde (mg/g Hb, A), plasma levels of oxidized low-density lipoproteins (B) and plasma levels of advanced oxidation protein products (C) in normal weight, overweight and obese children. $^{b}P < 0.001$ *vs* control and overweight groups. Results are expressed as mean ± SE. MDA: Malondialdehyde; ox-LDL: Oxidized low-density lipoprotein; AOPP: Advanced oxidation protein products.

stress generated by ROS. The present findings are concordant with prior reports on obese humans and animal models which found that in the early stages of obesity development there may be an initial elevation in antioxidant enzymes to counteract oxidative stress[31]. The depletion of the antioxidant activities in obese children may be attributed to the high production of ROS which may destroy these antioxidant enzymes[32-34]. In addition, the decrease of erythrocyte GSH level (Table 2), which is an essential cofactor for GSH-Px, may lead to reduction of GSH-Px activity in obese children[35,36]. Furthermore, the reduced activities of these antioxidant enzymes in obese children may be attributed to the decreased expression of their mRNA. This finding is confirmed by the studies of Li *et al*[37] and Furukawa *et al*[38] which showed that the mRNA expression levels of antioxidant enzymes, such as SOD, CAT and GSH-Px, decreased in WAT of obese mice. The excess production of MDA (Figure 1A) has additional toxic effects for antioxidant enzymes. MDA may modify the amino acid side chains and oxidation of thiol groups of these enzymes, resulting in a partial or complete loss of their activities and functions[39]. Fang *et al*[40] found that the Ox-LDL correlated negatively with SOD expression and they reported that the decreased activity of SOD may be attributed to excess production of ox-LDL which inhibits the expression of SOD.

GSH plays multiple roles in the cell, including being a free radical scavenger as a primary antioxidant defense[7]. The significant decrease of erythrocyte GSH content in overweight and obese children may be due to its increased turnover into its oxidized form (GSSG) through its detoxification of ROS and other peroxides to challenge the prevailing oxidative stress generated by ROS[41]. This is consistent with GSH function to scavenge oxidants by binding with them covalently[42]. Furthermore, the reduction in erythrocyte GSH content may be attributed to its use in the recycling of vitamin E and semihydroascorbic radicals and reduced oxidized molecules, such as lipid hydroperoxides[43]. In addition, a decrease in the GSH level in the red cells may result from depressed GSSG-R activity (Table 2)[44].

The common approach in the measurement of oxidative stress is the determination of MDA, a product of lipid peroxidation. Thus, the excess production of ROS with insufficient antioxidant enzymes in obese children may have a serious adverse effect on RBC membranes, resulting in lipid peroxidation enhancing production of MDA concentrations similar to our cases (Figure 1A)[38]. This finding is in agreement with the study of Ustundag *et al*[45] which showed the elevation of plasma MDA in smaller groups of obese children when compared with healthy controls.

The current study showed the increase level of ox-LDL (Figure 1B), the second approach in the measurement of oxidative stress in obese children. Increased levels of ox-LDL may be related to excess oxidative stress with lowered antioxidant defense[46]. This finding was demonstrated in 1992 by Parthasarathy *et al*[47] who reported that obese children and adolescents have higher levels of ox-LDL due to generation of ROS compared to a normal weight group.

AOPPs are considered reliable markers to estimate the degree of oxidant-mediated protein damage. The observed increases in AOPP levels in the present study (Figure 1C) suggest that proteins might be an important oxidative target of accumulation of oxidative stress in severe childhood obesity[48]. This argument has been confirmed by the study of Atabek *et al*[49] which found that AOPP levels were increased in obese children and adolescents.

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The antioxidant enzymes and the more important oxidatively modified macromolecules, *e.g.,* MDA, ox-LDL and AOPP, have not been completely explored in overweight and obese children because only data on antioxidant vitamin levels are available[11-13]. The current study aimed to evaluate the activities of SOD, CAT, GSH-Px,

GSSG-R, levels of MDA, ox-LDL and AOPP in Saudi overweight, obese and normal weight children to identify obese children who are at risk of complications.

COMMENTS

Background

Alarmingly, obesity and its complications have especially increased in the last three decades among pediatric populations, reaching epidemic proportions in developed and developing countries. Excess body fat expressed as body mass index (BMI) is highly correlated with systemic oxidative stress, which is considered an imbalance between the concentrations of reactive oxygen species (ROS) and the oxidative defense mechanisms. The oxidant-antioxidant status has not been completely studied in overweight and obese children. Therefore, the present study investigates oxidatively modified macromolecules, *e.g.*, malondialdehyde (MDA), oxidized low density lipoproteins (ox-LDL) and advanced oxidation protein products (AOPP), and antioxidants, *e.g.*, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-R) and reduced glutathione, in overweight and obese children.

Research frontiers

Studies on obese adults show that oxidative stress is related to BMI. Therefore, the research hotspot is to investigate this relationship in overweight and obese children by estimating oxidative damage products, *e.g.*, MDA, ox-LDL and AOPP, and antioxidant defenses, *e.g.*, SOD, CAT, GSH-Px, GSSG-R and GSH, which are studied as marks of oxidative stresses in obese children.

Innovations and breakthroughs

Due to the lack of availability of information regarding oxidative stress markers in obese children, the novelty of this work is the investigation of oxidative stress within this age group. The children were classified according to their BMI, the precise evaluation of which may be done by a combination of biomarkers that represent a different aspect of oxidative damage products. Examples of such are MDA, AOPP and ox-LDL, and antioxidant defenses, such as SOD, CAT, GSH-Px, GSSG-R and GSH.

Applications

In the future, these findings will be translated into clinical evaluation prior to the overt manifestation of diseases or to assess the benefits of treatment of obese children. In addition, the measurement of oxidant-antioxidant parameters may be useful in understanding the pathophysiology of obesity-related health effects.

Terminology

The authors' findings indicate that severe childhood obesity, represented by BMI, is associated with significantly increased AOPP and ox-LDL concentrations. In addition, the recognition of these changes early in childhood is important for preventing the long-term complications of obesity and targeting individual subjects who are particularly at risk. The authors' results may be helpful in increasing research to expand both prevention and therapeutic strategies for obesity to minimize oxidative stress in children.

Peer review

This is a good descriptive study in which the author did many experiments which illustrated the relationship between BMI and oxidative stress in obese children. The manuscript was interesting to read and the results are interesting. The data and the results in this paper are also very clear and will help the relationship between obese and oxidant-antioxidant status to be understood.

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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Saps M, Rosen JM, Ecanow J

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Volume 3 Number 2 May 8, 2014

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OBSERVATIONAL STUDY

X-ray detection of ingested non-metallic foreign bodies

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Abstract

AIM: To determine the utility of X-ray in identifying non-metallic foreign body (FB) and assess inter-radiologist agreement in identifying non-metal FB.

METHODS: Focus groups of nurses, fellows, and attending physicians were conducted to determine commonly ingested objects suitable for inclusion. Twelve potentially ingested objects (clay, plastic bead, crayon, plastic ring, plastic army figure, glass bead, paperclip, drywall anchor, eraser, Lego™, plastic triangle toy, and barrette) were embedded in a gelatin slab placed on top of a water-equivalent phantom to simulate density of a child's abdomen. The items were selected due to wide availability and appropriate size for accidental pediatric ingestion. Plain radiography of the embedded FBs was obtained. Five experienced radiologists blinded to number and types of objects were asked to identify the FBs. The radiologist was first asked to count the number of items that were visible then to identify the shape of each item and describe it to a study investigator who recorded all responses. Overall inter-rater reliability was analyzed using percent agreement and κ coefficient. We calculated P value to assess the probability of error involved in accepting the κ value.

RESULTS: Fourteen objects were radiographed including 12 original objects and 2 duplicates. The model's validity was supported by clear identification of a radiolucent paperclip as a positive control, and lack of identification of plastic beads (negative control) despite repeated inclusion. Each radiologist identified 7-9 of the 14 objects (mean 8, 67%). Six unique objects (50%) were identified by all radiologists and four unique objects (33%) were not identified by any radiologist (plastic bead, Lego™, plastic triangle toy, and barrette). Identification of objects that were not present, false-positives, occurred 1-2 times per radiologist (mean 1.4). An additional 17% of unique objects were identified by less than half of the radiologists. Agreement between radiologists was considered almost perfect (kappa 0.86 ± 0.08, $P < 0.0001$).

CONCLUSION: We demonstrate potential non-identification of commonly ingested non-metal FBs in children. A registry for radiographic visibility of ingested objects should be created to improve clinical decision-making.

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Key words: Foreign bodies; X-rays; Pediatrics; Radiographic phantom; Diagnostic imaging

Core tip: Foreign body (FB) ingestion is very common in children and results in numerous visits for acute medical evaluation. X-ray identification of FB location and retention is used to guide management decisions including performance of additional imaging studies or FB retrieval. We investigated whether non-metal FB were visible on X-ray using a radiographic phantom. Our results show that expert radiologists are potentially unable to identify ingested non-metal foreign bodies. Cre-

ation of a database to catalogue X-ray characteristics of ingested non-metal objects would enable clinicians to improve quality of care by reduction of false-negative tests and prevention of unnecessary procedures.

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INTRODUCTION

Foreign body (FB) ingestion in children was reported to United States poison control approximately 100000 times in 2010[1]. In children, the ingestion of FB is frequently not witnessed[2]. Unwitnessed FB ingestion poses a diagnostic challenge with important therapeutic implications[3,4]. Children are often too young or frightened to provide a reliable history. Children can remain asymptomatic despite ingestion of a potentially harmful FB. Even in the asymptomatic child, retention of an ingested FB may necessitate removal depending on type, location, or size. To solve this diagnostic and therapeutic challenge, physicians usually obtain X-ray studies (XR)[5]. XR is instrumental in cases of metal objects, however its utility is limited in cases of non-metal objects of unknown visibility. Despite limitations, XR is widely recommended as an initial diagnostic tool due to disadvantages of other imaging techniques (magnetic resonance imaging, computed tomography, and ultrasound) including cost, radiation exposure, and expertise/equipment required for performance and interpretation.

Although button batteries[6] and magnets[7] appropriately receive attention in the media and scientific literature because of harmful, sometimes fatal, ingestions, other objects also present significant risks. Case reports identify ingested non-metal materials including a plastic toy[8], pencil[9], tape[10], bottle cap diaphragm[11], ballpoint pen[12,13], toothbrush[14], eel vertebrae[15], twig[16] and other unusual objects[17,18] that may not be readily identifiable with routine XR. Ingestion of plastic toys can lead to intestinal obstruction[19] or other toxic effects of chronic retention due to plastic constituents[20]. Intestinal perforation also may result from ingestion of non-metal objects[21,22].

Confronted with the uncertainty of a possible FB, providers cannot make an informed and efficient decision. The provider may opt to rely on an XR result that provides false reassurance and potentially leads to otherwise preventable morbidity. Alternatively, the practitioner may distrust XR results and perform unnecessary diagnostic tests (*i.e.*, endoscopy) with increased risk to the child. Instruction of parents to screen or strain the child's stools for FB passage is inconvenient and may raise parental and child anxiety.

Despite high frequency of FB ingestion and importance of diagnostic radiography in guiding therapy, there are only case reports describing ability of XR to detect specific non-metallic FBs. Increased understanding of XR utility in diagnosis of non-metallic FBs is an initial step to improve clinical care. We conducted a study assessing non-metallic FB identification by XR.

MATERIALS AND METHODS

The primary aims of our descriptive study were to assess the ability of radiologists to detect non-metallic FBs through radiography and to determine the inter-rater agreement of detection. A secondary aim of our study was to create an easily reproducible model to assess visibility of ingested FB in children. Phantoms are frequently used for calibration and testing of imaging devices in radiology departments. For the purpose of this study we custom designed a water-equivalent phantom (WEP) to model the density of the child's body. WEPs are composite materials that allow simulation and testing of radiographic techniques without human radiation exposure. To encase foreign bodies, a 2 cm thick gelatin-in-water mold was prepared by dissolving 28 g of gelatin in 1 quart of tap water (Knox Gelatin, Kraft Foods, Inc., Tarrytown, NY). The gelatin slab was placed on top of a standard WEP (Gammex Solid Water, Laco, Inc., Chesterland, OH) to create a composite (gelatin/WEP) phantom.

Twelve unique items, some with "choking hazard" warnings, and two copies of one item were encased in the gelatin slab (clay, plastic bead, crayon, plastic ring, plastic army figure, glass bead, paperclip, drywall anchor, eraser, Lego™, plastic triangle toy, and barrette). Potential items for inclusion were determined by conducting focus groups with nurses, gastroenterology fellows, and attending physicians. The items included were selected due to wide availability and appropriate size for accidental pediatric ingestion. Copies of one item, a plastic bead, were used to evaluate reliability of our model. A metallic paperclip was used as a positive control. The slab with encased objects was placed on an 18 cm thick WEP to create a 20 cm thick composite phantom representative of a 20 cm thick child. A single XR was obtained of the composite phantom with encased objects (Axiom Multix M, Siemens United States Corp., Washington, DC, United States, 70 kVP, 39 mAs, phototimer technique, table-top Bucky grid).

Five board certified radiologists, each with ten years or greater experience, and blinded to the number and identity of the items in the gelatin slab, were shown a single radiograph. The radiologist was first asked to count the number of items that were visible on the radiographic image. After recording the number of items identified, each radiologist was asked to identify the shape of each item and describe it to a study investigator who recorded all responses.

Statistical analysis

Overall inter-rater reliability was analyzed using percent agreement and κ coefficient. κ coefficient values < 0 indicate poor, 0-0.2 slight, 0.21-0.4 fair, 0.41-0.6 moderate,

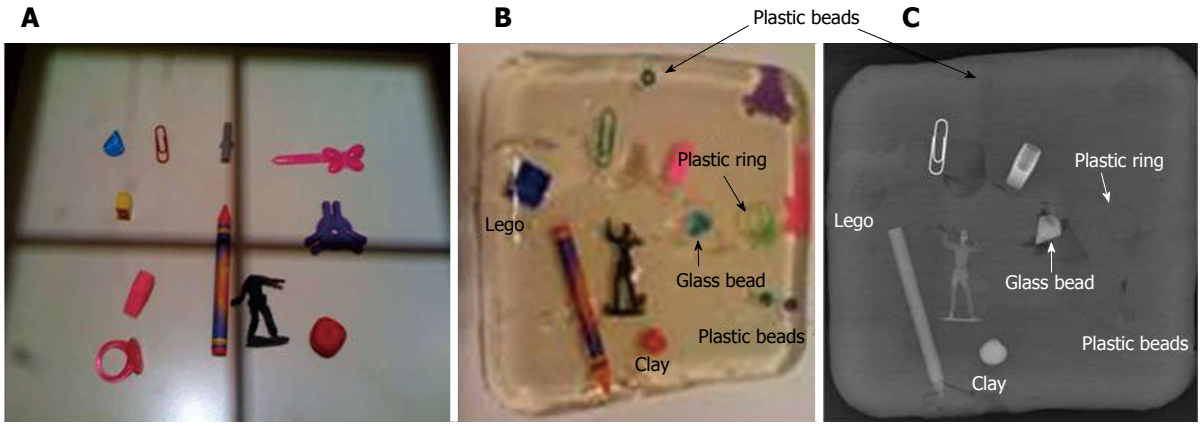

Figure 1 Images of foreign objects. A: Color photograph before embedding in gelatin; B: Color photograph after embedding in gelatin; C: Plain X-ray taken with objects embedded and placed on water-equivalent phantom.

Table 1 Summary of object detection by radiologists

No.	Rad 1	Rad 2	Rad 3	Rad 4	Rad 5	Object
1	+	+	+	+	+	Army figure
2	+	+	+	+	+	Clay lump
3	+	+	+	+	+	Crayon
4	+	+	+	+	+	Eraser
5	+	+	+	+	+	Glass diamond bead
6	+	+	+	+	+	Paperclip
7	-	+	-	-	+	Ring
8	+	-	-	-	-	Drywall anchor
9	-	-	-	-	-	Lego
10	-	-	-	-	-	Pink barrette
11	-	-	-	-	-	Plastic bead
12	-	-	-	-	-	Plastic bead 2
13	-	-	-	-	-	Plastic bead 3
14	-	-	-	-	-	Triangle purple plastic
x	+					"Half violin"
x		+				"Irregular elongated rectangle"
x			+			"Irregular opacity"
x				+		"Irregular opacity"
x				+		"Linear lucency"
x					+	"Irregular lucency"
x					+	"Irregular opacity"

Rad: Radiologist; +: Positive identification of object denoted; x: No object/ false positive.

0.61-0.8 substantial, and 0.81-1 almost perfect agreement, respectively. We calculated *P* value to assess the probability of error involved in accepting the κ value. This study was performed without the use of human or animal subjects.

RESULTS

Fourteen objects had color photographs taken before and after embedding in gelatin, and plain X-ray taken after embedding (Figure 1). Each radiologist identified 7-9 of the 14 objects (mean 8, 67%). Six unique objects (50%) were identified by all radiologists and four unique objects (33%) were not identified by any radiologist (round plastic bead, Lego™, pink barrette, and purple triangle). Identification of objects that were not present, false-positives, occurred 1-2 times per radiologist (mean 1.4). An additional 17% of unique objects were identified by less than half of the radiologists. Agreement between radiologists was considered almost perfect (kappa 0.86 ± 0.08, $P < 0.0001$) (Table 1).

DISCUSSION

This is the first published study assessing visibility of non-metallic objects using an inexpensive, simple, easily reproducible model that represents a child's body density. Gelatin slabs have variable distribution of embedded air bubbles that may help simulate the presence of fat in the child's body. The excellent agreement found in our study among experienced radiologists validates (face value) the model. If the accuracy and utility of our model is confirmed in larger studies, it could potentially be used to predict the visibility of FB in children. The fact that some objects could not be seen by any radiologist suggests that such an object may not be visible if ingested by children. More than half of the items were either not detected or detected inconsistently, raising the potential for unnecessary diagnostic XR or missed opportunity for intervention in patients. Toy ingestions represent almost 7% of all phone calls to poison control in children less than 5 years of age in the United States[1]. Some of the most popular toys used by children such as Legos™ could not be visualized by any radiologist. Regardless of whether a given ingested object presents significant risk, parental (and primary care provider) anxiety often leads to repeated patient evaluation.

The results of our study underscore the need for an easily accessible FB registry denoting XR visibility. The creation of such a registry could be instrumental in helping radiologists and emergency room physicians in diagnosis and management. Enhancing confidence in diagnosing the presence of an ingested FB could facilitate the physician's medical plan and alleviate parental anxiety. The European Registry on Upper Aerodigestive Tract Foreign Body Injuries in Children (Susy Safe)[23] serves as a successful model with data input at the point of

care by all member institutions. Susy Safe data has demonstrated the need for ongoing collection of information to identify modifiable risk factors in toy design[24] as well as the ongoing necessity of integrative preventative strategies[25,26]. Our proposed registry should be internet-accessible and include standardized data fields for radiographic characteristics of FB that are lacking in the Susy Safe registry.

Imaging modalities other than XR for detection and characterization of ingested foreign bodies are rarely used in clinical and research settings. Ultrasonographic techniques show promise and are radiation free[27], but they require a skilled technician at the point of care and specialized equipment. Other modalities including contrast XR, computed tomography[28], and magnetic resonance imaging (MRI) may be helpful in specific circumstances, but involve increased radiation exposure (except MRI), cost, and complexity.

Limitations of this study include the utilization of a model, rather than clinical data, and a small panel of objects subjected to radiographic identification. However, the use of a model simulating the child's body helps circumvent the ethical considerations of obtaining repeated XR in children.

Our study demonstrates the potential inability to detect ingested non-metallic FBs in children using XR. It also shows that while agreement among radiologists is excellent, some objects are inconsistently or never detected. Some of the objects not detected are common, present in many households and easily accessible to children. We propose a model to assess the visibility of non-metallic FB by XR. Future studies including a larger pool of objects should be conducted to confirm our findings. The creation of an easily accessible FB database should be considered.

COMMENTS

Background

Foreign body (FB) ingestion in children is common. X-ray (XR) is a simple diagnostic test that may assist with identification and localization of ingested FB. However, it is not clear whether non-metal objects are radiopaque. This leads to significant difficulty in utilizing XR to guide treatment decisions.

Research frontiers

The study proposes a simple, clinically relevant imaging model that could be used to predict FB visibility in children.

Innovations and breakthroughs

Despite high frequency of FB ingestion and importance of diagnostic radiography in guiding therapy, there are only case reports describing ability of XR to detect specific non-metallic FBs. The study assesses non-metallic FB identification by XR.

Applications

A similar model can be used at the point of care to predict XR visibility of an ingested foreign body. This could limit unnecessary procedures, or more rapidly and accurately indicate whether endoscopic or surgical retrieval is needed.

Peer review

The authors studied the utility of XR in identifying non-metallic foreign bodies and inter-radiologist agreement in identifying non-metal foreign body using custom designed water equivalent phantoms, and concluded that the potential inability to detect ingested non-metallic foreign bodies in children using XR. This paper is well-written and has interesting and important findings.

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TOPIC HIGHLIGHT

Keith Collard, PhD, Associate Professor, *Series Editor*

Transfusion related morbidity in premature babies: Possible mechanisms and implications for practice

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Abstract

Many premature babies, especially those with a low birth weight are given multiple transfusions during their first few weeks of life. The major serious complications of prematurity include bronchopulmonary dysplasia, with lesser incidences of retinopathy of prematurity, intraventricular haemorrhage, and necrotising enterocolitis. Many studies have shown correlations between the receipt of blood transfusions and the development of these conditions, but little is known of the underlying pathophysiology of this relationship. Recent studies are beginning to provide some answers. This review examines recent findings with regard to the influence of preparation and storage of paediatric packed red blood cell units on heme, iron, and oxidative status of the units and relates these to the ability of the premature baby to deal with these changes following the receipt of blood transfusions. Paediatric packed red blood cell units are a potential source of heme, redox active iron and free radicals, and this increases with storage age. Haemolysis of transfused red blood cells may add further iron and cell free haemoglobin to the recipient baby. Premature babies, particularly those with low birth weight and gestational age appear to have little reserve to cope with any additional iron, heme and/or oxidative load. The consequences of these events are discussed with regard to their contribution to the major complications of prematurity and a novel hypothesis regarding transfusion-related morbidity in premature babies is presented. The review concludes with a discussion of potential means of limiting transfusion related iron/heme and oxidative load through the preparation and storage of packed red blood cell units and through modifications in clinical practice.

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Key words: Blood transfusions; Premature babies; Iron; Heme; Oxidative stress; Storage lesion; Complications of prematurity

Core tip: Many premature babies, especially those with a low birth weight are given multiple transfusions during their first few weeks of life. Studies have shown correlations between the receipt of blood transfusions and the development of the major complications of prematurity. Little is known of the underlying pathophysiology of this relationship. This review examines novel potential mechanisms which are related to the changes that occur in iron, heme and oxidative status in paediatric packed cell units during preparation and storage, and in the ability of the premature baby to deal with these changes following receipt of blood transfusion.

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INTRODUCTION

Many premature babies, especially those with a birth

weight of less than 1000 g are given multiple transfusions during their first few weeks of life[1,2]. The major serious complications of prematurity include bronchopulmonary dysplasia (BPD), with lesser incidences of retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), and necrotising enterocolitis (NEC). Many studies have shown correlations between the receipt of blood transfusions and the development of these conditions[1,3-16]. Few have been able to provide any strong evidence that the receipt of blood transfusions is an independent risk factor in the development of these conditions[3,4,6,9,15,17], although some more recent studies have provided better evidence of transfusion being an independent risk factor for NEC[13] and IVH[9]. This lack of consistency is probably related to the multifactorial nature of these conditions, the multifactorial nature of the consequences of the receipt of packed cell transfusions[18], and the fact that smallest and sickest babies are those most likely to receive blood transfusions[19]. This makes it difficult to tease out the relative risks of the many factors involved[20] even with sophisticated multiple regression analysis[2,4,19]. Despite this, some explanations have been proposed to account for the relationship between the receipt of blood transfusions and some of the consequences of prematurity. Many of these involve transfusion mediated iron induced factors such as infection and oxidative stress[19], changes in immune function[21] and also other factors such as changes in nitric oxide (NO) mediated vasodilation and responsiveness[22,23].

This paper attempts to determine more about transfusion-related morbidity in premature babies by relating some of the recently observed changes which occur in paediatric packed cell units during preparation and storage to the particular physiology of the premature baby. The result has provided strong evidence to suggest that a major contributor to transfusion-related morbidity in these babies is the enhanced level of non-protein-bound iron, heme and oxidative stress in the paediatric packs and the limited ability of the premature baby to deal with a transfusion-mediated iron, heme and oxidative load. It is a relatively new concept that does not preclude other aspects of the "storage lesion" also contributing[24,25], but at present it appears to be a good marriage between the known physiology of the premature baby and the effects of packed red blood cell transfusions.

BLOOD TRANSFUSION AND CLINICAL OUTCOME IN PREMATURE BABIES

The relationship between blood transfusion and NEC has been linked to possible adverse immunological consequences of the receipt of blood and the timing of this with feeding[11,21,26], although this should be less of a problem when using leukoreduced blood preparations[27]. Transfusion related gut morbidity is however a multifactorial condition related to a dynamic balance of immune, infectious, vascular, angiogenic and mechanical mediators of brush border integrity[28]. It also appears to be related to particular changes occurring at 31-32 wk postconceptional age, irrespective of postconceptional age at birth. There is evidence that the older the storage age of the blood transfused the more likely NEC is to develop[17], and oxidative stress has been mentioned as a potential factor[28]. However, the complexity of the condition has not permitted a clear understanding of the relationship between the receipt of blood and the development of NEC. Also, many babies who receive blood transfusions do not develop NEC[15], indicating that those in which the relationship is seen may have other underlying factors that predispose towards developing NEC following transfusions[29,30]. Certainly the smaller the birth weight the more likely the baby is to develop transfusion associated NEC[17]. Thus the receipt of blood may be one factor in a complex multifactorial condition.

The link between IVH and the receipt of blood may be related to volutrauma and damage to the weak blood vessels in the germinal matrix[10]. This may be further exacerbated by the loss of NO from erythrocytes during storage[22,31] which would impair capillary vasodilation to accommodate the donated erythrocytes[9]. It should be noted however, that not all studies support this model of erythrocyte mediated vasodilation[9,32]. However, it has been shown that blood which had been stored for up to 42 d has a progressive vasoinhibitory effect which is mediated not by scavenging NO or loss of NO, but by inhibiting endothelial NO production in the recipient[23]. Thus there is evidence of disruption of NO vasodilatory mechanisms in the recipient following the receipt of stored red blood cells. Again, it should be noted that not all babies who develop IVH receive blood transfusions, and many babies who receive blood transfusions do not develop IVH[9]. Yet again there is a subset of vulnerable babies in which the receipt of blood becomes a risk factor. Current views on the link between transfusion and IVH does not include iron-induced oxidative stress. However, it could play a major role in events subsequent to the haemorrhage as blood (potentially rich in redox active iron) entering the extracellular compartment is likely to contribute to iron-induced oxidative damage to the cells of the developing brain[33,34].

While volutrauma may also contribute to ROP, most studies suggest that iron overload and associated oxidative stress may be a major player in ROP[12,35]. Increased iron load due to post-transfusional red blood cell (RBC) breakdown and associated oxidative stress has been suggested[12,35]. In addition, enhanced O_2 delivery to the developing retinal vasculature following transfusion with adult RBC's may impair the function of the growth factors which regulate vascularisation of the retina[12]. Again low birth weight and respiratory distress also appeared to be independent risk factors for the development of ROP[35], highlighting the multifactorial nature of the condition.

Though not so well investigated, transfusion-related iron overload and resulting oxidative stress has been suggested as a potential mechanism linking transfusion to the development of BPD[19,36-38]. The relationship between blood transfusion and BPD may be related to the finding that babies with BPD were usually smaller, required more

ventilator support and required more blood sampling leading to iatrogenic anaemia. Consequently more blood transfusions would be required to replace that removed by sampling[38]. This suggests a potential consequence of very low birth weight rather than a direct cause of BPD. However, the receipt of blood and associated complications caused by it may exacerbate a condition developing from other causes. A major factor in the development of BPD is endotracheal infection[4]. A recent study in critically ill adults has shown that transfusion with blood stored for more than 14 d is associated with increased bacterial infection[39]. Since iron availability is essential for bacterial colonisation[40], and the level of non-transferrin bound iron in paediatric packs rises significantly throughout storage (around 6 μmol/L on day 14, around 15 μmol/L on day 35, compared with plasma levels of around 0.3 μmol/L in healthy adults[41]), transfusion mediated iron promoted bacterial infection may be involved in the development of BPD.

Thus, in summary, there is a reasonable amount of evidence to support of increased risk of developing the major complications of prematurity following the receipt of blood transfusions in some premature babies. As indicated above, potential mechanisms which have been proposed to account for this relationship include disruption of NO mediated vasodilation[22-24], immune dysfunction[11,21,42], and transfusion mediated iron and oxidative load[19,41,43]. These are not necessarily mutually exclusive and have not been established as independent risk factors in all cases. Interaction between these factors probably occurs. For example, structural changes in the stored red blood cells which influence the deformability and survival of red blood cells post transfusion, lead to the build-up of extracellular haemoglobin which can have major effects on NO availability[44-47], and also provides a potential source of heme and iron[48] for iron mediated pathology and immune modulation[42].

Particularly interesting are the findings that the link between blood transfusions and the complications of prematurity are more prevalent in the smaller birth weight babies, and that one complication may be associated with the presence of another in this group[49]. This may indicate that this subset of premature babies may require specific attention with regard to transfusion practice.

The awareness of the potential risks of receiving blood transfusions has led to a number of studies and changes in clinical practice to try to limit the use of blood transfusions in the neonatal intensive care unit as a means of improving clinical outcome[50-58]. These procedures are beyond the scope of this review.

Recent findings have strengthened the idea that transfusion-mediated iron and oxidative load may play a major role in some of the complications of prematurity. These findings include factors involved in the preparation, storage and use of packed red blood cell units in premature babies and the ability of the baby to deal with potential adverse consequences of the receipt of blood. This review will investigate the possible link between transfusion-mediated iron overload and oxidative stress and the ability of the premature baby to deal with such a situation, and the implications with regard to the development of the complications of prematurity.

PREPARATION OF PAEDIATRIC PACKED CELL UNITS FOR TRANSFUSION

In order to try to understand the potential mechanisms of any relationship between the receipt of blood transfusions and clinical outcome some knowledge of the procedures involved in the preparation of packed cell units is required. Paediatric packed red blood cell units are prepared from adult blood. One unit of adult blood usually anticoagulated with citrate is spun down to yield the red blood cells. Blood for paediatric use is usually filtered to remove the majority of leucocytes. The majority of plasma is removed and replaced by additive solution to provide a haematocrit of 55%-60%. Various different additive solutions are used but they all tend to contain various amounts of dextrose, adenine, phosphate, mannitol and occasionally citrate, either residual from the original anticoagulation or added[59]. The additive solutions are designed to provide anticoagulant and buffering capacity and a source of metabolic energy for RBCs. In addition, mannitol and adenine act as preservatives to allow the storage of RBCs up to 35 d for paediatric use and 42 d for adult use[60] in the United Kingdom. These latter substances stabilise the RBC membrane and ensure adequate 2,3-diphosphoglycerate and ATP availability within the RBCs. Each adult unit is then divided to provide 6-8 paediatric packs of 40-50 mL each. While these additives have been designed to help preserve RBC integrity and shelf life, the removal of plasma has significant implications with regard to iron and iron-induced oxidative stress. The replacement of plasma with additive removes the major iron binding proteins and extracellular antioxidants from the final preparation[41]. This provides the opportunity for the build up of redox active iron in the extracellular medium and the potential to drive iron mediated oxidative damage to the RBCs, and to induce iron mediated oxidative damage to the baby post transfusion[61]. The purpose of this review is to evaluate the possibility that the contribution of the receipt of blood transfusions to the development of the complications of prematurity may reside, to some extent, in poor iron status in the paediatric packs and the consequences alluded to above. This will require an understanding of changes in iron and oxidative status of paediatric packs during storage, and the extent to which the baby might cope with increased iron and oxidative load post transfusion.

THE INFLUENCE OF STORAGE ON THE IRON AND OXIDATIVE STATUS OF PACKED CELL UNITS AND THE RELATIONSHIP BETWEEN STORAGE AND CLINICAL OUTCOME

Traditionally, only blood stored for less than 7 d had

been deemed acceptable for neonatal transfusions[62]. Because some babies require frequent transfusions, the number of different donors that an individual baby may be exposed to could be high. This was considered as potentially detrimental to the baby and wasteful with regard to resources[63]. For these reasons, the use of small volume paediatric packs prepared from a single donor (as described above) was adopted as standard use[63]. This allowed the packs to be stored up to 35 d and ensured that the baby should only receive blood from a single donor. The move from the use of fresh blood to stored blood required some understanding of the consequences of RBC storage on the status of the blood and the influence of older stored blood on clinical outcome. A number of studies have shown adverse relationships between the storage age of the blood used in transfusion and clinical outcome[64,65]. This relationship holds whether the blood is transfused to critically ill infants[64,65] or adults[66]. However it should be noted that not all studies support this contention. To some extent this may be related to the failure to address all the potential confounding variables[20,67-70]. The potential adverse effects of storage on the biochemistry and validity of stored erythrocytes has been given the term "the storage lesion"[52,71]. The controversy surrounding this contention, and the need to improve our understanding of the influence of storage on clinical outcome is illustrated by the development of two current large scale studies looking at the influence of storage age on clinical outcome in critically ill adults[72], and in premature babies[73]. Both these studies are prospective studies using clearly defined storage ages and outcome measures. The initial results of the latter study showed that babies who received blood of an average storage age of 5.1 d did not have an improved outcome compared to babies who received blood stored under the current standard procedure which averaged out in this study at 14.6 d[74]. However, as discussed later, it is more likely to observe adverse outcomes in babies who have received blood stored for greater than 14 d. This is difficult to study prospectively because of the ethics of randomly assigning blood stored for more than 14 d to a group of babies knowing it might compromise outcome.

The involvement of iron and oxidative status in the storage lesion has received little attention, despite the potential adverse influence of the procedures involved in the preparation of packed cell units on the iron and oxidative status of the units. This can have consequences on the viability of erythrocytes, and the behaviour of haemoglobin within the erythrocytes and on iron bioavailability[75,76].

A number of studies have indicated that transfusion mediated iron overload may contribute to morbidity and mortality in some situations[78], and that this may be increased as a function of the storage age of the blood transfused. Other studies have indicated that this may be mediated by iron released from RBCs during storage as a result of oxidative damage to RBC membranes and haemoglobin[76]. The first study to show that iron is indeed lost from packed RBCs during storage was that of Marwah *et al*[79]. In this study little non-transferrin-bound iron (NTBI) was seen in the extracellular medium surrounding the stored RBCs over the first 10 d of storage. Thereafter it rose steadily up to 28 d storage. In our laboratory using leucoreduced paediatric packed cell units iron was already detectable in the extracellular medium on arrival from the blood transfusion centre (3 d post donation) and then rose linearly to a level of 34 μmol/L after 35 d storage[41,43]. Moreover a high percentage was in the potentially damaging NTBI form. This suggests that some release of iron was occurring as a result of damage occurring during preparation[80], and that some changes occur very rapidly in the first few days of storage[81]. It is not believed that the filtration process used to remove the leucocytes is the cause of the initial haemolysis, but other factors such as shear stress, exposure to anticoagulants, exposure to additive solutions and contact with the plastic material in the bags[80]. Leukoreduction may improve the storage of RBCs[82]. Packed cell units used in neonatal intensive care units are routinely leucoreduced. In our studies the rise in extracellular iron and NTBI with storage is associated with a gradual increase in malondialdehyde (MDA) over the first 21 d of storage with a steeper rise from 21 d to 35 d. Similar findings have recently been reported by Stark *et al*[83], and the findings reflect chances in cellular MDA during storage[84]. The appearance of Hb in the extracellular medium parallels the rise in MDA. This indicates that lipid peroxidation in the RBC membrane may contribute to the loss of iron and Hb. In addition to the rise in iron with storage, there is also a large increase in heme in the extracellular milieu[48,85] with the pattern of increase running parallel to that of MDA. MDA is marker of lipid peroxidation. It is well known that oxidative stress, normal ageing and aerobic incubation lead to the release of free chelatable iron from Hb within erythrocytes[77,86]. There is evidence that iron released within erythrocytes can mediate oxidative damage to the cell membrane, leading to haemolysis and the release of Hb[76]. This may be further oxidised to produce superoxide, methemoglobin and free iron[86,87]. Methemoglobin is relatively unstable and will readily release the heme moiety from the heme pocket[77]. Further oxidation of the heme molecule leads to the release of free iron.

The small amount of residual ascorbate present in the extracellular medium was mostly in the oxidised form and fell dramatically from 21 to 35 d[41]. Thus the lack of antioxidant protection contributes to oxidative damage to the cell membrane and iron binding proteins such as Hb[76,88,89]. The findings from our laboratory support the previous findings of Karon *et al*[90] who noted similar findings with regard to the development of adverse effects in the membranes of stored RBCs including enhanced lipid peroxidation and build-up of extracellular Hb. Thus the evidence suggests that oxidatively mediated haemolytic changes to RBC membranes and damage to iron binding proteins leads to release of iron from the RBCs into the extracellular medium during storage. The

finding that these changes may occur early in storage, coupled with the lack of antioxidant protection suggests that the released iron can generate more free radical species and potentially initiate a vicious cycle of oxidative damage and iron release. This scenario is supported by the pattern of changes in heme and MDA, with the rise in both parameters initially being gradual over the first 14-21 d of storage and then more rapidly during the latter stages[48,85]. An alternative, but related hypothesis, to account for iron-induced adverse effects has been presented by Hod *et al*[91,92]. Their work using a murine model of transfusion with stored blood has suggested that extravascular haemolysis of transfused RBC's by macrophage-mediated phagocytosis leads to a pro-inflammatory response which is associated with increased circulating NTBI. Furthermore, reactive oxygen species induced by NTBI may mediate cytokine production and promote the pro-inflammatory response. These studies were followed up by investigating the situation in healthy human adults[93] and premature babies[83]. Both studies reported transfusion mediated increases in NTBI, and this was associated with increased oxidative stress in the premature babies[83]. However, neither study was able to demonstrate the increased appearance of pro-inflammatory cytokines. Thus the involvement of NTBI in transfusion related immunomodulation in premature babies has yet to be established[83], although immune modulation mediated by other components that increase during storage (such as heme) may occur[42].

The enhanced NTBI levels seen post-transfusion may also promote bacterial infection, a factor of relevance to conditions such as chronic lung disease of prematurity[4]. It is also suggested that heme present in transfused blood may promote nosocomial infection through its effect on the innate immune system[42].

To develop these ideas further, some understanding of how well the premature baby is able to handle an enhanced transfusion-mediated iron load is required.

THE ABILITY OF THE PREMATURE BABY TO DEAL WITH TRANSFUSION MEDIATED IRON AND OXIDATIVE LOAD

Because of the physiological importance of iron and the potential toxic effects of free iron the body is equipped with a very precise homeostatic mechanism to regulate iron bioavailability[61]. The function of the major components of this system in premature babies has been investigated in a number of studies. Studies into iron status and iron binding and transporting proteins in premature babies are complicated because the levels of these proteins may be influenced by oxidative stress and free iron levels[94,95]. Although results are not always conclusive, the results suggest that both the levels and binding capacity of transferrin in the plasma of premature babies is low[96-99]. The most recent study[100] showed that premature babies had elevated iron and percentage iron binding levels compared to normal reference values. This was particularly so in male babies, who tend to show a greater degree of morbidity than their female counterparts[101,102]. In addition to the specific iron binding to transferrin, albumin may also play a role as an antioxidant by binding free iron and limiting the ability of iron to generate free radicals[103]. The ability of albumin to bind iron seems to be particularly important as a defence mechanism against iron induced oxidative damage[60]. Studies have reported significantly lower serum albumin levels in premature infants compared with term infants[104]. Serum albumin in premature infants is particularly susceptible to oxidative damage[105] which would further limit its ability to bind iron. Caeruloplasmin, which converts iron to the form necessary to bind to transferrin may also be low in prematures. Serum hepcidin concentrations were lower in preterm infants than full term babies[106]. This was believed to reflect the lower total iron stores of premature babies. There was a good correlation between hepcidin levels and the levels of ferritin and erythropoietic activity. However, it was not possible to detect a significant correlation between hepcidin and transferrin levels or transferrin saturation, despite this population of babies having low transferrin levels and high transferrin saturation[96,100]. Post-transfusional changes in serum hepcidin have not been studied in premature babies, but oral iron supplementation in low birth weight infants led to increases in circulating hepcidin[107] as did blood transfusions in adults with thalassemia[108]. Should this also occur in premature babies it may enhance iron sequestration in macrophages and limit iron-induced toxicity. However, more studies are required to explore this further. Thus it appears that some aspects of the regulation of hepcidin activity by iron status appear to be functional in premature babies, but how well hepcidin is able to be upregulated in response to a transfusionally mediated enhanced iron load has yet to be elucidated. In addition to potential iron overload, the large rise in heme provides a second potentially toxic mediator in stored packed red blood cells. Free heme has both pro-oxidant and pro-inflammatory activity[109,110] and many other potentially toxic activities[111]. The potential toxicity of heme is limited by the presence of the heme binding protein hemopexin[110]. Thus an adequate availability of hemopexin is necessary to prevent the toxic effects of heme. Premature babies have very low levels of hemopexin[112] which makes them vulnerable to the effects of transfusion mediated heme overload.

The results of studies on the antioxidant status of premature babies are largely in agreement. It appears that they have limited antioxidant defences to protect against circulating free radicals[3,4,113-116]. With regard to the low molecular weight antioxidants ascorbate, urate and possibly glutathione in serum and bronchoalveolar lavage fluid in premature babies, studies have shown that the levels of these antioxidants fall during the first week of life and recover over the next few weeks[3,4,113,117]. Premature babies who require blood transfusions will receive their first transfusion, and possibly the majority of their treatments within

the first week of life. Thus the receipt of blood, with the possibility of generating excessive free radicals, coincides with a period when antioxidant protection through the low molecular weight antioxidants is falling. Studies on the major enzymic antioxidants in premature babies have shown reduced levels of glutathione peroxidase and superoxide dismutase[116]. Furthermore, the ability to upregulate pulmonary superoxide dismutase in response to inspired O_2 and free radicals is impaired in premature babies[118] and animal models[119,120]. Similarly, peroxiredoxin does not appear to upregulate in preterm baboons in response to high inspired O_2 concentrations[121].

In summary, the premature baby appears to be poorly equipped to deal with any form of heme and iron overload and subsequent iron induced oxidative stress. Consequently the premature baby is likely to be at risk of transfusion-related heme, iron and oxidative overload.

POSTRANSFUSIONAL CHANGES IN IRON AND OXIDATIVE STATUS IN PREMATURE BABIES

A limited number of studies have examined iron status in premature babies following the receipt of blood transfusions. Assessment of premature babies at 35 wk post menstrual age indicated that 50% of babies who received more than 3 erythrocyte infusions were iron overloaded at that stage of their care irrespective of when they received the transfusions[122]. Earlier studies[123] showed no difference in plasma bleomycin-detectable iron (NTBI) between babies who did or did not receive blood transfusions. However, the total number of samples containing bleomycin-detectable iron was significantly greater in babies who developed BPD compared to those who did not. Later studies by Hirano *et al*[124] showed that bleomycin-detectable iron was present in 30% of premature babies before transfusion and rose to 80% after transfusion. Measurement of total iron in premature babies (post mortem) showed that those who received more than 100 mL of blood had a higher total serum iron level than those who received less than 100 mL[125]. More recently, Dani *et al*[126] found that the plasma level of NTBI increased significantly following blood transfusion, but that this was not associated with any evidence of increased oxidative stress in plasma up to 3 h after transfusion. In contrast to this, studies in our laboratory showed that pulmonary oxidative stress increased following blood transfusion[2], and in babies that received more than one transfusion oxidative stress increased after each transfusion. The most recent study[83] supports our findings showing increases in blood MDA following blood transfusion in premature babies. NTBI also increased and was correlated to the storage age of the packed cells transfused. Positive correlations between NTBI and MDA were also reported. The effect of transfusion on NTBI was transient, as was the increase in pulmonary MDA seen in our studies following transfusion[2]. The major difference between the studies of Collard *et al*[61] and Stark *et al*[83] and those of Dani *et al*[126] was the age of the babies studied. In the study by Dani *et al*[126] the gestational age of the babies studied was almost 10 wk greater than those studied in our study, and also older than those studied by Stark *et al*[83]. The antioxidant capacity, which increases with gestational age and birth weight[127], may have been sufficiently well developed to deal with the pro-oxidant effect of iron in the babies studied by Dani *et al*[126] but not in those in the other two studies. This interpretation is supported by the findings of Minghetti *et al*[128] who showed that the antioxidant capacity of weight disparate twin babies was lower in the smaller babies and associated with enhanced lipid peroxidation. This adds to the previous data that indicated that there may be a subset of smaller lower gestational age babies which are particularly vulnerable to transfusion related morbidities. Little is known about post-transfusional changes in free heme in premature babies. This lack of knowledge needs rectifying urgently.

INTERIM SUMMARY, IMPLICATIONS FOR FUTURE STUDIES AND FOR BLOOD TRANSFUSION PRACTICE IN THE NEONATAL INTENSIVE CARE UNIT

Premature babies, particularly those with low birth weight and gestational age appear to have little reserve to cope with any additional iron, heme and/or oxidative load.

Paediatric packed red blood cell units are a potential source of heme, redox active iron and free radicals, and this increases with storage age. Haemolysis of transfused red blood cells may add further iron and cell free haemoglobin to the recipient baby.

The link between the receipt of packed cell transfusions and the complications of prematurity may be due to some extent to the additional heme, iron and oxidative load caused by transfusion. This relationship may be particularly significant in low birth weight and gestational age babies.

In order to develop this idea further or to refute these suggestions there is an urgent need to conduct appropriate clinical studies. Clinical studies have shown that blood stored for an average of 14 d does not cause any additional complications when compared with fresh (5 d storage) blood[72]. This fits well with the findings of the biochemical studies which indicate that changes in the parameters discussed above progress slowly over the first 14-21 d and then change more rapidly. The effect of blood stored for longer periods has yet to be established in this group of babies. This would be difficult to study by means of a prospective randomised trial because it would be unethical to randomly subject babies to receive blood stored for more than 14-21 d. An alternative strategy would be to conduct a study in which all babies in a neonatal intensive care unit receive blood stored for

less than 14 d for an appropriate period (say 2 years) and compare outcomes with data from the previous 2 years in which blood stored for up to 35 d was routinely used.

Perhaps the best way of obtaining data in a shorter time span would be to conduct a retrospective study using clinical records from neonatal intensive care units in which the storage age of the blood used was recorded. This would allow the categorisation of data into groups in which blood beyond 14 d storage could be compared with those receiving blood less than 14 d old. This approach has recently been suggested by Flegel[129]. Because of the multifactorial nature of the clinical conditions under investigation, all confounding variables will need to be recorded and a detailed multifactorial analysis conducted in order to tease out the relative risk factors of all the variables[4] including storage age of blood used.

There are also some modifications to the preparation of the paediatric packed red cell units which might be investigated in an attempt to limit the availability of redox active iron and free radicals in the units. This could include the addition of haptoglobin, hemopexin, apotransferrin and/or antioxidants to the additive fluid. The effects could be easily evaluated *in vitro* and in animal models, but transferring the findings to clinical applications would be difficult in premature babies without detailed studies on the safety and efficacy of such preparations in appropriate human subjects.

In the short term, until the outcome of further clinical studies is known, it might make sense to limit the storage age of blood given to premature babies to 14 d. This is supported by studies which indicated that, from a biochemical and molecular standpoint, the parameters defining the integrity of saline, adenine, glucose mannitol stored leucoreduced red blood cells may be acceptable up to 14 d of storage, but then decline[84], and clinical studies have shown no additional adverse effects of blood stored for a mean age of 14 d compared to fresh blood[74]. A cut-off point of 14 d is supported by many other studies. Blood stored for more than 14 d was associated with multiple organ dysfunction and prolonged stay in the paediatric intensive care unit[65]. In adults, the incidence of bacterial infection increased following transfusion with blood stored for more than 14 d[39], and the incidence of mortality almost doubled in those patients receiving blood stored for more than 14 d compared to those receiving blood stored for 7 d[130]. The most detailed investigation conducted on adults undergoing cardiac surgery[131] showed conclusively that blood that had been stored for more than 14 d was associated with significantly increased risks of post operative complications, in-hospital mortality and poorer long term outcome compared to patients receiving fresher blood. The study investigated large numbers of patients and provided strong statistical power. Thus the biochemical and clinical data support the view that to reduce the incidence of morbidity and mortality in patients requiring transfusions with packed red blood cells, the blood should be stored for no more than 14 d. Limiting storage age to 14 d would have clear logistic and cost implications. Limiting the maximum shelf life from 35 d to 7 d is predicted to result in a 50% decrease in the number of available units and a fourfold increase in the number of units outdated each year[132]. An expiration date of 14 d would have a significant impact on hospital reserves, and would require a substantial increase in collections to preserve hospital stocks[132]. These figures are based on blood group matched adult units. The situation regarding O negative paediatric packs for use in premature babies would be more disruptive. It may make sense to conduct a small pilot study to fully evaluate the feasibility of such a change in practice.

In addition as there appears to be a subset of premature babies which are particularly vulnerable to the adverse effects of transfusion, we should already be giving the freshest blood available to the smallest and youngest babies. The need to re-evaluate transfusion practice with regard to the storage age of the blood has recently been suggested for blood transfusion in adults[129]. There is perhaps a more urgent need to do the same in premature babies who are probably at a greater risk of transfusion mediated morbidity than adults.

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REVIEW

Neurodevelopmental outcome in congenital diaphragmatic hernia: Evaluation, predictors and outcome

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Abstract

To review the reported neurodevelopmental outcome of congenital diaphragmatic hernia (CDH) survivors, identify important predictors of developmental disabilities, and describe the pathophysiological mechanisms contributing to adverse outcome. A Medline search was performed for English-language articles cross-referencing CDH with pertinent search terms. Retrospective, prospective, and longitudinal follow-up studies were examined. The reference lists of identified articles were also searched. Neurodevelopmental dysfunction has been recognized as one of most common and potentially most disabling outcome of CDH. Intelligence appears to be in the low normal to mildly delayed range. Neuromotor dysfunction is common during early childhood. Behavioral problems, hearing impairment, and quality of life related issues are frequently encountered in older children and adolescence. Disease severity correlates with the degree of neurological dysfunction. Neurodevelopmental follow-up in CDH children should become standard of care to identify those who would benefit from early intervention services and improve neurological outcomes.

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Key words: Congenital diaphragmatic hernia; Extracorporeal membrane oxygenation; Neurodevelopment; Bayley scale of infant development; Wechsler Preschool and Primary Scale of Intelligenc; Developmental disabilities; Quality of life; Autism

Core tip: Neurodevelopmental dysfunction has been recognized as one of the most common comorbidity in congenital diaphragmatic hernia (CDH) and survivors. Disease severity impacts on neurological dysfunction. Neurodevelopmental follow-up in CDH children should become standard of care to improve neurological outcomes.

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INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a common anatomical anomaly in which there is herniation of the abdominal viscera into the thoracic cavity due to an incomplete closure of the pleuroperitoneal membrane. It is estimated to occur in approximately 1 in 2500 live births.

Over the past several decades, the postnatal survival rate at tertiary centers has improved with reported rates of 70% to 90%[1,2]. Improved survival rates appear to be the result of advances in surgical technique, neonatal intensive care, extracorporeal membrane oxygenation (ECMO), and the widespread implementation of lung

preservation ventilation strategies. While improving short-term morbidity and mortality are important goals, more recent research has focused on long-term outcome as equally important primary outcomes[3-9].

Neurodevelopmental and neurofunctional impairments constitutes one of the most common and most significant morbidity in CDH[10]. There is an increasing concern for considerable delays in various neurological domains: cognitive, motor, language, and visuospatial skills, hearing impairment, and behavioral development[5,10-16].

The purpose of this review is to comprehensively examine the available literature that report on cognitive, motor, and behavioral development in these patients; to identify important predictors of developmental disabilities; and to summarize the pathophysiological mechanisms contributing to adverse outcome.

COMPREHENSIVE OUTCOME STUDIES

Short-term outcome (≤ 36 mo)

Several studies have evaluated the short-term outcome in CDH children and demonstrated that the neurodevelopmental deficits seen in CDH patients during infancy appears to be comparable to the data reported in infants born with other severe congenital malformation (*e.g.*, congenital heart disease, giant omphalocele, bronchopulmonary dysplasia, preterm infants, and children with deletion 22q11 syndrome)[17-21]. A study by Van Meurs *et al*[22], showed that 25% of CDH children that required ECMO had mild deficits, while seventeen percent showed significant delays. Comparable data were reported by D' Agostino *et al*[23] showing average, mild delays and severe delays in 54%, 23% and 23%, respectively. Bernbaum *et al*[14] reported an increased incidence of periventricular leukomalacia and intraventricular hemorrhage (26%), and seizure activity (5%) in CDH survivors. More recently, Cortes *et al*[24] assessed neurocognitive outcomes at 24 mo of age and showed that more than 50% of CDH survivors demonstrated cognitive delays, while nearly 40% were found to have neuromotor dysfunction. Chen *et al*[25] evaluated motor skills in 13 CDH children during early childhood; nearly 80% of participants showed impairments.

In a pilot study of 41 CDH patients enrolled in the Pulmonary Hypoplasia Program at the Children's Hospital of Philadelphia, Danzer *et al*[12] found at a median of 2 years cognitive and language skills within the average, mild delays, and severe delays in 49%, 36%, and 15% respectively. Motor skills were average in 46% while 23% had mild deficits, and 31% had severe neuromotor deficits. Comparable data were recently reported by Wynn *et al*[26] in a multicenter prospective follow-up study of 48 CDH survivors at 24 mo of age.

Intermediate-term outcome (37 mo to ≤ 5 years)

Neurological assessments during school age generally are relatively stable and more predictive of long-term outcome. However, data on preschool neurodevelopmental outcomes is sparse. Nijhuis-van der Sanden *et al*[27] studied neuromotor skills in 32 CDH survivors. Thirty-eight percent had normal scores. A total of 34% had mild delays and sixteen percent had significant problems[27]. In a similar study, van der Cammen-van Zijp *et al*[28] found various degrees of neuromotor delays in 42%.

In one of the largest prospective follow-up study, Danzer and associates[11] followed 60 CDH patients. The majority of CDH children have more favorable neurodevelopmental outcome at early preschool age and performed well within the average range. Twenty-two percent of evaluated CDH patients had borderline scores. Eleven percent showed significant delays in at least one domain[11].

Long-term outcome (above 5 years of age)

As children get older, it is possible to assess and study a broader range of neurological function, however analogous to the data shown above, only a few reports focus on school age and adolescence outcome in CDH patients. Bouman *et al*[29] studied 11 CDH patients at 10 years of age. Below average IQ scores were found in nearly half of the population. Jacobson *et al*[16] compared 15 CDH survivors to age-matched controls at a follow-up of 13 years. Although the mean neurodevelopmental and functional scores were similar between groups, nearly one-fourth of the CDH children scored below average. Significant delays were found in 13%.

Similarly, Rasheed *et al*[16] reported the data of CDH survivors that required ECMO either after or before surgical closure of the diaphragm defect. IQ scores for children that had surgery before ECMO were within the average range, while children that underwent ECMO first had below average scores. Overall neurofunction scores were similar between groups.

Recently, Tureczek *et al*[30] evaluated 33 CDH children without ECMO support at 9 years of age using the WPSSI-Ⅲ and the Movement Assessment Battery for Children 2nd edition. Although they report that the overall neurocognitive scores were not significantly different than population norms, they reported increased rates of motor dysfunction[30].

The long-term implications of these findings throughout adulthood are uncertain. Continued outcome research is warranted as these children progress through school, since lower academic scores, learning disabilities, and/or the presence of behavioral problems, might be associated with increased risk of school failure which in turn may lead to poor social skills, low self-esteem, disinhibition, and delinquency. Of note, the improvements in perinatal care of CDH neonates in the past decade coupled with the improved understanding of the pathophysiological sequelae associated with CDH may make it difficult to extrapolate the reported data in currently school-age children to newborns born in the past decade. It is likely that current CDH survivors will have better neurological outcomes than those born just a generation or two before.

Longitudinal outcome

Similar to the lack of long-term outcome data, there is a paucity of longitudinal assessments. Longitudinal evaluations are important as it has been acknowledged that several neurodevelopmental disabilities may be transient, while others may continue to evolve later in life when more complex cognitive and executive performances are required.

To date, only three studies reporting on the longitudinal neurodevelopmental outcome in CDH. Gischler *et al*[31] evaluated 12 CDH infants every six months for the first two years of life. Cognitive and language scores at 6 and 24 mo were average. Neuromotor scores slightly improved from the low-average range to the average range.

Friedman *et al*[32] followed 23 CDH survivors during the first three years of life. Of the 17 children noted to have neurofunctional problems at three years of age, 13 had already variable degrees of neurofunctional impairments at one year.

In 2013, Danzer *et al*[10] longitudinally evaluated the neurodevelopmental outcome of 47 CDH children in the first three years of age. During the study period, BSID-Ⅲ neurodevelopmental and motor scores improved in 19% and 37%, respectively. In spite of the performance improvements, the number of CDH patients with mild to severe delays in at least one neurodevelopmental area was greater than expected for the general population[10]. Seventy-two percent of the children scored within the average range for all three domains, while 17% were delayed in either neurodevelopmental or neurofunctional outcome, 11% had delays in all domains, and 6% remained severely delayed.

HEARING IMPAIRMENT

Hearing impairment, mainly sensorineural hearing loss (SNHL) can be viewed as another type of neurodevelopmental complication. SNHL appears to be a progressive phenomenon; with reported incidences between 0% to 100%[24,33-42]. In a recent report, Partridge and associates[37] followed 112 CDH patients. Interestingly, SNHL was found in approximately 3%, a rate comparable to the prevalence of SNHL in graduates from neonatal intensive care units for other problems (2%-6%). Unexpectedly, they found a high incidence (34%) of abnormalities in auditory brainstem response and/or behavioral audiometry consistent with conductive hearing loss.

Although hearing impairment in CDH has been attributed to a number of risk factors, the pathophysiology remains poorly understood. For example, need for ECMO, severe hypoxia, acidosis, duration of mechanical ventilation and NICU stay, and the prolonged exposure to ototoxic drugs have all been reported as risk factors[24,33-37]. Furthermore, genetic predisposition to hearing loss and cumulative noise exposure have also been postulated as potential causes of SNHL in CDH patients. Additional studies are necessary to define their role in the CDH population. In general, infants with hearing impairment are at increased risk for delayed language acquisition, poor social development, and impaired academic achievement. Early identification and appropriate intervention may be critical in minimizing adverse effects and optimizing developmental outcomes.

QUALITY OF LIFE

Although, quality of life (QoL) assessment has emerged as an essential outcome measurement in many high-risk patient populations (*e.g.*, congenital heart disease, extremely low-birth weight children), few studies have evaluated QoL in the CDH population. Poley *et al*[13] studied 111 CDH children. Preschool CDH patients had lower scores in five of the thirteen domains tested. Adolescents demonstrated considerable deficits in several areas of every day functioning. No differences were found between young CDH adults and the control population.

In a study of 69 adults with CDH, Koivusalo *et al*[43] found lower QoL scores and the frequency of attaining higher educational levels (*e.g.*, college) in 25% of the study population. Bouman *et al*[29] assessed the emotional outcomes and that 36% of CDH children may have depressive problems.

Chen *et al*[44] studied the QoL of 53 CDH children at a median age of 8 years and found that ongoing clinical problems translated into lower functional status, particularly in overall general health and interpersonal functioning. Further research is warranted to delineate associations between specific aspects of neurodevelopmental outcome and QoL and to identify neurologic impairments that may be improved through early intervention. By characterizing the relationship between disease complexity, neurodevelopmental morbidity, and QoL, health care professionals and caregivers may be able to significantly improve the lives of CDH children and ensure their future success.

RISK FACTORS FOR ADVERSE NEUROLOGICAL OUTCOME

Based on the available outcome data, various risk factors for adverse neurodevelopmental sequelae have been identified[8,9,12,23-25,31,32,45]. For example, position of the liver one of the most important factors of survival. In one of the largest series of isolated left-sided CDH patients, Hedrick *et al*[2] demonstrated that neonates with intrathoracic liver position had a mortality of approximately 55% and 80% required ECMO. Of note, Danzer *et al*[12] reported that more than two-thirds of CDH children with prenatally diagnosed intrathoracic liver position had delayed neurodevelopmental function.

Several studies have reported a survival advantage of right-sided CDH compared to left-sided defects[46,47]. This improved survival of right CDH children must be cautioned by a high prevalence of associated problems, suggesting that the higher incidence of neurologic deficits these children may be in part due to the survival of

extremely sick right CDH patients.

The need for ECMO is associated higher risk of neurological impairments[12,14,23,27-29,35,48,49]. Whether the increased incidence of adverse outcome associated with ECMO indicates a more severe form of CDH or a reflection of ECMO-associated complications continues to be under discussion. In general ECMO is reserved for the sickest newborns[50,51]. ECMO therapy may also be linked to neurological impairments due to the need for anticoagulative therapy, development of intracranial hemorrhage, and alteration of intracranial blood flow secondary to the necessary ligation of the carotid artery. The type of ECMO modality used may impact function. Historically, venoarterial (VA) ECMO has been used. Recent studies suggest that venovenous ECMO may be as useful as VA ECMO with a lower prevalence of sequelae[52,53].

The need for a patch to repair the diaphragm or the need for oxygen beyond 30 d may also play and important role in neurological outcome[17,23,24,46].

In addition to the above mentioned potentially modifiable predictors, many independent risk factors of adverse neurodevelopmental outcome are not modifiable, such as innate patient-related variables, including associated malformations, genetic syndromes, the higher than expected incidence of autism and autism spectrum disorder[17]. The apparent link between CDH and autism spectrum risk is of concern. If more CDH children are diagnosed with autism in the future, one should consider including them as part of outcomes. Moreover, parental education and social-economic status, which are also not modifiable factors, are also associated with adverse outcome. Stolar *et al*[53] reported that low-level maternal education correlates with the incidence of delays. Wynn *et al*[26] expanded on these initial findings and showed that not only maternal education, but also paternal education and household income less than $30000 were associated with lower neurodevelopmental and functional scores.

PATHOPHYSIOLOGIC MECHANISM

While the abovementioned predictors support the concept that disease severity correlates with the severity of neurodevelopmental problems, the mechanisms remain poorly understood. Several reports suggest that children with CDH have a higher incidence of cerebral abnormalities than the general population[54-57]. Hunt *et al*[55] found a disturbingly high incidence of brain abnormalities in CDH newborns on postnatal magnetic resonance imaging studies. Danzer *et al*[56] found that the development of the brain in CDH neonates might be delayed. They also found that 18% of infants studied had periventricular leukomalacia, as well as delayed closure of the cerebral opercula in 14%. Although the etiology of central nervous system injury in CDH patients is almost certainly multifactorial, changes in cerebral circulation are common and appear to play a pivotal role[56]. In normal fetal brain development, the formation of the cerebral cortex begins at about 6 wk gestation with the formation of the ventricular zone of the dorsal and ventral germinal matrixes, followed by a well-orchestrated sequence of structural changes including the gradual appearance of deep primary and more superficial secondary cortical infolding, neuronal migration and arborization, synaptogenesis, programmed cell death, oligodendrocyte maturation, and extensive reorganization of synaptic connections during the second half of gestation[58-63]. Beginning in the third trimester, myelination of the cerebral hemispheres accelerates[64,65]. These important processes during fetal brain development place an escalating demand on the cardiopulmonary system for delivery of oxygenated blood. The observed brain abnormalities in CDH might be in part caused by prolonged impairment of cerebral oxygen delivery. Of note, in children with congenital heart defects, the brain receives lower levels of oxygen-saturated blood from the right ventricle as a consequence of disordered fetal circulation[66,67]. In CDH fetuses, the left ventricle is one-third smaller and the left ventricular output is reduced[68-70]. These alterations may affect cerebral perfusion and compromise cerebral development. Of note, Buesing *et al*[71] showed that cerebral blood flow is altered in CDH survivors[71,72]. In addition to a prenatal insult, CDH neonates are exposed to the potential risk of hypoxia/ischemia, emboli, reactive oxygen species, acidosis, neuro-modulating drugs and inflammatory microvasculopathy before and after surgery, all of which may affect the white matter maturation and in turn neurodevelopment[59,63,73-77].

CONCLUSION

Infants and children with CDH often have significant neurodevelopmental and neurofunctional sequelae compared with population norms. Some of the identified early developmental abnormalities may improve over time. Identifying deficits early and providing early physical, occupational, and academic interventions may help to improve neurological morbidities before additional disabilities evolve and optimize long-term academic achievements. The American Academy of Pediatrics[18] has established follow-up guidelines for CDH survivors after discharge to highlight the importance of monitoring the developmental problems in this high-risk population throughout infancy and childhood.

Future research should focus on intervention strategies that not only reduce the pulmonary sequelae in CDH, but also in improving prenatal hemodynamics and cerebral blood flow to optimize brain development and improve outcome. Further, robust and longitudinal studies that incorporate advanced neuroimaging techniques and comprehensive assessments are warranted to further improve outcomes in CHD survivors.

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REVIEW

Preventing medication errors in neonatology: Is it a dream?

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Abstract

Since 1999, the problem of patient safety has drawn particular attention, becoming a priority in health care. A "medication error" (ME) is any preventable event occurring at any phase of the pharmacotherapy process (ordering, transcribing, dispensing, administering, and monitoring) that leads to, or can lead to, harm to the patient. Hence, MEs can involve every professional of the clinical team. MEs range from those with severe consequences to those with little or no impact on the patient. Although a high ME rate has been found in neonatal wards, newborn safety issues have not been adequately studied until now. Healthcare professionals working in neonatal wards are particularly susceptible to committing MEs due to the peculiarities of newborn patients and of the neonatal intensive care unit (NICU) environment. Current neonatal prevention strategies for MEs have been borrowed from adult wards, but many factors such as high costs and organizational barriers have hindered their diffusion. In general, two types of strategies have been proposed: the first strategy consists of identifying human factors that result in errors and redesigning the work in the NICU in order to minimize them; the second one suggests to design and implement effective systems for preventing errors or intercepting them before reaching the patient. In the future, prevention strategies for MEs need to be improved and tailored to the special neonatal population and the NICU environment and, at the same time, every effort will have to be made to support their clinical application.

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Key words: Medication errors; Drug safety; Adverse events; Prevention; Newborn

Core tip: Although a high medication error (ME) rate has been found in neonatal wards, newborn safety issues have not been adequately studied until now. Healthcare professionals working in neonatal wards are particularly susceptible to committing MEs due to the peculiarities of newborn patients and of the neonatal intensive care unit environment. Current neonatal prevention strategies for MEs have been borrowed from adult wards, but many factors such as high costs and organizational barriers have hindered their diffusion. The present article reviews current issues related to MEs in Neonatology and discusses the strategies to prevent them and to improve the safety of newborns.

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INTRODUCTION

Since 1999, following the publication of the United States Institute of Medicine (IOM) report "To err is human: building a safer health system", the problem of patient safety (PS) has drawn particular attention. This report has revealed that the problem of accidental patient injury was serious and that adverse events occurring during hospitalization were responsible for killing or damaging a large number of patients. It reported that from 44000 to

98000 deaths due to medical errors occurred every year in United States hospitals, while over 7000 deaths were due to medication errors (MEs) in or out of the hospital[1].

Both individuals and society are burdened with medical error consequences. Physical and psychological uneasiness, delayed hospital discharge, and costs in terms of human lives weigh heavily on patients. The productivity reduction of workers, higher hospital costs, and increasing insurance premiums are some of the most important social costs. Accordingly, it is of utmost importance to investigate medical errors more extensively and to implement efficacious interventions to control and prevent them. Many efforts have been dedicated to the prevention of medical errors in adult wards, and to the improvement of adult PS. Conversely, PS is an unfrequently addressed and inadequately studied topic in neonatological literature, although the medication administration error rate in the Neonatal Intensive Care Unit (NICU) is quite high, reaching values up to the 15%[2], and adverse drug events occur three times more frequently in newborns than in adults[3].

The present article reviews the various issues related to MEs in Neonatology and discusses the strategies to prevent them and to improve the safety of newborns. The importance of creating a culture of safety in the neonatal ward is also emphasized.

PATIENT SAFETY: A SHORT HISTORY

"Every hospital should follow every patient it treats long enough to determine whether the treatment has been successful, and then to inquire 'if not, why not' with a view to preventing similar failures in the future". These words had been published in a pamphlet in 1914 by Ernest Amory Codman, an American surgeon that can be considered the forerunner of the modern preventive medicine. He introduced a new patient care system, which included an "End result card" containing demographic data, diagnosis, treatment and outcome of each treated patient. Codman believed that sharing mistakes and experiences recorded with this system in a public forum would allow physicians to learn from each other's mistakes, improving quality of future patient care. Today this system represents the basis for many quality improvement plans[4].

Since the 1940s, high-risk industries such as aviation started to develop an appropriate approach to reduce the risk of human errors, designing a system able to intercept them or to provide means able to reduce their consequences in case of non-detection. This approach has led to the development of risk management programs that have proven to be effective in reducing risks and their consequences.

In the 1950s, risk management programs were introduced in United States hospitals based on the observation that physicians, like pilots, are required to have high level performance in a high risk environment and to make decisions under pressure; moreover, they are both aware that their mistakes might cost human lives. At first, risk management programs were focused on preventing certain accidents to patients (*e.g.*, patient falls, and the oversight of sponges inside patients during surgery). Since the 1980s, individual and system factors leading to erroneous decisions of the clinical team were investigated, and programs to prevent medical errors were improved.

CONCEPTUAL APPROACHES TO HUMAN ERROR: THE MEDICAL ERROR

The IOM[1] defined medical errors as "the failure of a planned action to be completed as intended or the use of a wrong plan to achieve an aim". In general, medical errors include all errors occurring in the health-care system: they may be made by all clinical team professionals during all stages in the care process, from diagnosis to treatment.

The consequences of this type of error burden society primarily with hospital costs resulting from the increased need of monitoring, of diagnostic tests, and of drug administration to control their effects. Furthermore, medical errors can lead to delayed hospital discharge, temporary or permanent patient disability, and cost of lives. Other important consequences are represented by the loss of patient trust in the healthcare system and the increase of insurance premiums. Finally, there are social consequences of medical errors such as the absence of children from school, the absence and less productivity of workers, and the worsening of population health status. Therefore, studying the causes of medical errors and developing the prevention strategies for reducing them are nowadays considered important targets to improve PS.

Two different approaches have been proposed to address the problem of human error: the person approach and the system approach. The first approach concentrates on individual errors resulting from the subjective mental state, such as inattention, negligence, and forgetfulness. In contrast, the second approach focuses on the working environment and conditions as the origin of errors[5].

The combined effect of "active failures" by individuals and "latent failures" in the system has been found to be responsible for error occurrence. In the hospital setting, active failures may involve every professional in the team such as doctors, pharmacists, and nurses. By contrast, latent failures are weaknesses which usually lie dormant in the system until they combine with active failures or a triggering event, thus creating an accident opportunity that makes them manifest. Faulty information management, stressful environment, inadequate training of personnel and ineffective communication systems represent some examples of latent failures. According to the psychologist Reason, latent errors are those "waiting to happen"[6]. The analysis of these errors is very important as it can reveal how to change a system and make it safer[7]. According to the Swiss Cheese Model, every accident is not a consequence of a single error but is the

Table 1 Stages of the medication pathway in the hospital setting

Stage	Healthcare professional involved
Decision on appropriate medicine	Physician
Prescription	Physician
Review of prescription	Pharmacist
Preparing, dispensing and checking the medicine	Pharmacist
Delivery of medicine to the ward (or home)	Pharmacy staff or nurses
Preparing to administer medicine	Nurse
Administering medicine to patient	Nurse
Recording medicine on patient chart	Nurse
Monitoring patient response	Nurse

end result of a chain of factors, accidentally placed in a proper sequence that penetrates or bypasses defences, barriers and safeguards, and results in an incident[5].

MEs: DEFINITION, TYPOLOGY AND SEVERITY

Medication is the commonest intervention within the health-care system. The errors resulting from medication may cause iatrogenic injuries which can be prevented.

ME is "any preventable event that occurs in the process of ordering, transcribing, dispensing, administering, or monitoring a drug, irrespective of whether an injury occurred or the potential for injury was present"[8]. Since MEs may happen at any step of the medication pathway process, they can involve every professional of the clinical team (Table 1).

The commonest types of MEs concern dose, time, rate (drug delivered more slowly or faster than prescribed), technique of preparation, route, and administering technique; additionally, omission errors and others (wrong site, wrong patients, unauthorized drug, *etc*.) have been reported[9].

Lack of information about patient and drug can lead doctors to make errors in the phase of drug ordering[7]. Calculation errors are the most frequently described prescribing errors[3,9,10]. They include miscalculation of the dose, incorrect expression of the measurement unit, wrong decimal point placement, and indication of mistaken drug administration rate[11]. Difficult-to-read handwritten physician orders is another important factor increasing ME risk and patient harm[12]. Hartel *et al*[13] found a generally bad readability of the handwritten prescriptions, rated as bad in 52%, and unreadable in 4% of cases. They documented a good readability of the handwritten prescriptions only in 2% of them, and a moderate one in 42%.

The most frequently documented type of MEs is over-dose, the least is reduced interval[3]. The absence of a specified administration route has been found to be the commonest error in the phase of transcription[10]. Pharmacists are mainly prone to make dispensing errors both in reviewing medical prescriptions and in diluting stock solutions to administer extremely low doses[14].

Nurses normally intervene at the end of the pharmacotherapy process, preparing and administering drugs. Therefore, they have the opportunity to detect errors made by doctors during the prescribing step, and to intercept them before they reach the patient[15]. Furthermore, nurses should be specifically trained to follow the Six Rights of medication administration, paying attention that the right medication is administered to the right individual, in the right dose, at the right time, *via* the right route, with the right documentation[16]. Recently, other items have been added to the six rights: in particular, nurses also have to pay attention to the right reason for the drug to be administered, the medication levels, and the date of expiry[17].

MEs have been classified both on the basis of their severity and on the basis of their causes. Six severity levels of errors were identified by the American Society of Hospital Pharmacists, ranging from level 1-"No injury" to level 6-"Mortality"[9]. MEs have been categorized into two main groups according to their cause: errors of commission and errors of omission. The first group includes all the errors due to the execution of an order not required, not needed or wrongly applied. The second type of errors happen when a drug is not prescribed or when an order necessary for the health of the patient is not applied[18]. A commission error occurs when a nurse violates one or more of the Six Rights of medication administration, while an omission error occurs whenever a prescribed medication is not administered to the patient[16].

MEs IN THE NEONATAL WARD

The reported rates of MEs in pediatric care vary greatly depending on different factors, including the setting of the study and the detection method used. Researchers usually quantify MEs using incident reports or chart reviews. Generally, as compared to incident reports, chart reviews are able to identify more errors.

Selected literature data on the epidemiology of MEs in hospitalized neonates and children are summarized in Table 2[8,19-25]. It should be noted that the wide variability in study design, clinical setting, detection method and study period prevents any comparison of ME rate across studies.

In general, newborn infants are more exposed to MEs and their adverse effects than adults due to their intrinsic characteristics. In particular, NICU patients have been shown to be more frequently exposed than hospitalized adults to potentially harmful errors[14]. Small size, physiological immaturity, limited compensatory abilities[26], rapid changes in weight and body surface area, and objective barriers to communication with the caregiver[9] are the main distinctive features of newborns, leading them to be more susceptible to negative effects resulting from MEs. In particular, newborns exhibit liver and kidney immaturity leading to a reduced drug metabolism and excretion, and thus they are particularly prone to more serious outcomes resulting from MEs[27]. Other pharmacokinetic processes are also immature in newborn patients, particu-

Table 2 Epidemiology of medication errors in hospitalized neonates and children: Selected studies

Ref.	Study design	Clinical setting	Detection method	Study period	Type of medication studied	MEs per 1000 pt-days	MEs per 100 admits	MEs per year
Raju *et al*[19], 1989	Prospective study	NICU, PICU	Incident/error reports	4 yr	All types	8.80	14.70	-
Vincer *et al*[20], 1989	Prospective study	NICU	Incident/error reports	2 yr	All types	13.40	-	-
Ross *et al*[21], 2000	Retrospective review	Ward, NICU, PICU	Incident/error reports	65 mo	All types	0.51	0.15	-
Kaushal *et al*[8], 2001	Prospective cohort study	Ward, NICU, PICU	Chart reviews	6 wk	All types	157	55	-
Frey *et al*[22], 2002	Prospective survey	NICU, PICU	Incident/error reports	1 yr	All types	-	-	284
King *et al*[23], 2003	Retrospective cohort study	Tertiary care pediatric hospital	Incident/error reports	6 yr	All types	4.49	-	-
Sangtawesin *et al*[24], 2003	Retrospective review	Pediatric hospital	Incident/error reports	15 mo	All types	-	1	-
Manias *et al*[25], 2014	Retrospective clinical audit	Ward, NICU, PICU	Incident/error reports	4 yr	All types	6.58	1.96	-

NICU: Neonatal intensive care unit; PICU: Pediatric intensive care unit; ME: Medication error.

larly in those born prematurely. In fact, differences in pH and in emptying time of the stomach may influence absorption phase, while high body water contents and low serum protein concentration may affect drug distribution.

Furthermore, the scarcity of data about pharmacokinetics, dosing, clinical use, efficacy, and safety of many medications in the newborn results in the frequent use of unlicensed or off-label drugs in the neonatal population. It was documented that approximately 10% of prescriptions in NICU were for unlicensed medications, while 55% were off-label[28]. As compared to licensed drug use, unlicensed use in the neonate was found to be more often associated with MEs[29].

Further possibilities of MEs in neonates result from the lack of concentrations and dosage forms appropriate to neonatal administration, the need to calculate individualized doses, the narrow therapeutic margin of many drugs, and the need of accurate and appropriate delivery systems (*i.e.*, pumps).

In addition, patient misidentification errors have been frequently documented in the NICU. The inability of NICU patients to take part in the identification process, the similar appearance of these patients in the first days of life that makes them not easily distinguishable, and the loss or removal of wrist identification bands are the main factors contributing to this type of error[30].

MEs are more common in the particular NICU environment, potentially resulting in patient injury[31]. In particular, they have been found to account for approximately 50% of the iatrogenic complications in neonatal intensive care[32]. The NICU is a complex system, where the environment is often chaotic with multiple unplanned admissions of critically ill patients requiring intensive care. In such complex systems, dedication, training, and vigilance of staff are insufficient to prevent errors[14]. Many factors have been found to influence the intrinsic risk of MEs, such as intensity of workload, understaffing, handoffs among health care providers, poor communication within the team, day shift, poor knowledge of the procedures, inappropriate use of technology, inadequate training, and absence of consciousness of errors[7,31,33]. Inexperience represents an additional risk factor for MEs. New staff make MEs more often because prescriptions are commonly written by junior doctors who may be less skilful in using medications[33]. Other factors related to caregivers such as inattention, distraction, haste, and fatigue principally affect the susceptibility to error[7].

Recently, the level and type of MEs in NICUs and neonatal units have been investigated, documenting that 37.8% of the nurses committed 1-2 MEs in a 6-mo period. The most frequent errors during injectable drug use were found to concern the time of drug administration, pharmaceutical calculation, and the ignorance of drug interaction in case of simultaneous prescription of more than one drug. Errors in pharmaceutical calculations and in medication dose were the most frequently observed in non-injection medication use[27]. Other studies documented that drug administration at the wrong time is the most frequent administration error[16,34,35]. A literature review by Chedoe *et al*[36] identified dose errors as the most frequent type of ME; they were found to result from an inexact recorded patient weight, a wrong unit of measurement, an incorrect recording of dosage regimen, or an erroneous placement of decimal point during dose calculation. Kunac *et al*[37] applied a technique named Failure Mode and Effects Analysis to the NICU medication process in order to identify high risk processes and to develop system improvement actions. This technique is used in industry to evaluate system safety and identify potential errors before they happen. The Authors documented that the most potential errors occur during drug prescription and preparation. Wrong setting of infusion pumps, incorrect doses, time and route of administration were the more frequently identified errors during administration step[37].

PREVENTION STRATEGIES FOR MEs IN NEONATOLOGY

Promoting a culture of safety has to be considered the most important tool to prevent MEs. A work environment where the culture of safety is widespread, and PS is considered more important than efficiency and produc-

tivity, can be defined a high-reliability organization, able to guarantee PS. The safety culture of an organization depends on many factors, namely group and individual values, competencies, perceptions and behavior models, that decisively influence ability and commitment to a safety management[38].

Since the 1950s, an increasing consciousness about the need to prevent MEs started spreading. In 1999, the IOM defined PS as "freedom from accidental injury", and established that it was a priority in improving quality of care. This esteemed institution laid out a comprehensive strategy and drew up a concise list of recommendations, offering a road map toward a safer health system[1]. Today, PS is an emerging concern of politicians, managers and caregivers. Measuring newborn PS is important in preventing MEs and helps to improve NICU safety, because it allows to propose changes and to monitor both the effects of PS interventions and the safety trends over time. The best PS measure is represented by the rates of events, that are mainly obtained from reporting, direct observation, chart review, and automated methods[39].

Recently, a list of alert events, including death or severe lesions due to drugs, has been drawn up. These events are called "sentinel events" as they are indicative of a malfunctioning in the healthcare system that requires immediate research and action[18].

Many intervention procedures have been developed to decrease the risk of MEs, and it is essential that all NICU staff are involved in the prevention of them[14,40].

Two different generic strategies have been proposed. The first strategy consists of identifying human behavioral factors resulting in errors and redesigning all the work in the NICU in order to minimize them; the second one suggests to design and implement dependable systems for preventing errors or intercepting them before reaching the patient[31].

Although system deficiency or failure is the primary source of errors, individual healthcare professionals' behavior also plays a role in the occurrence of MEs. Therefore, education and training of caregivers are considered an important step in the ME prevention policy.

Training programs are firstly aimed at improving communication competence between healthcare professionals and their patients and at building and reinforcing team communication ability. Training courses should also be held to provide caregivers with instructions and practice in performing mathematical calculations for drug dosage and patient monitoring during therapy administration. Recently, Campino *et al*[41] have studied the effects of a multidisciplinary education intervention on the number and type of MEs made in a NICU in the prescribing phase. The investigators documented that the implementation of this strategy led to a significant reduction of this type of errors from 20.7% to 3%, probably due to a behavior modification of doctors in the prescribing phase, and the spreading of a PS culture among health professionals. Another recent study has investigated the effects of an educational program on the rate and severity of some NICU MEs. The program consisted of several theoretical and practical lessons directed at nurses, about the preparation and administration of the most used drugs. The error rate decreased after the intervention period from 49% to 31%, although remaining significantly high. The authors concluded that this typology of intervention is able to reduce the error rate in medication preparation and administration. However, it is not sufficient alone to reach an adequate medication safety[42].

The Italian Society of Neonatology[43] drew up a practical guide aimed at reducing the risk of MEs in newborns. It consists of a formulary and a software program that provide neonatologists with a useful tool able to describe drug characteristics, the administration route and drug interactions, and to calculate the right prescription. It allows the creation of a personal file for each patient, reporting name, date of birth, weight and gestational age. The software program is able to work offline and can be updated over time[43]. Future studies are required to assess the effectiveness of this computerized support system.

Another strategy that was proven to reduce MEs is represented by the full-time presence of a dedicated clinical pharmacist in the NICU. This professional performs a daily review of medical prescriptions, suggests therapy changes, provides pharmacokinetic monitoring, educates and informs NICU staff and patients and helps in discharge planning[32].

Many efforts have been made to draw up detailed recommendations to prevent physician prescription errors which are due to unclearly legible prescriptions. It has been underlined that, in the prescription, physicians have to report any appropriate information on the patient (name, date of birth, weight, *etc*.). Furthermore, medication name, dose, quantity to be dispensed, administration route and frequency, therapy duration, and name of prescriber have to be clearly reported[44]. In case of the prescription written by physician assistants, the printed name, address, phone number, and signature of the supervising doctor will also have to be included. An additional control on medical prescriptions is made by nurses verifying dosage calculations, documenting all prescriber verbal orders, and repeating the orders back to him/her to verify them. Nurses' duties also include the verification of patient identity before giving the drug, and the administration of all doses at planned times[44]. For these reasons, nurse training should include a specific pharmacological education. An important step in the strategy of ME reduction regards clinical pharmacists in that they are responsible for the preparation and dispensing of prescribed drugs[44].

One of the most popular methods to investigate the processes involved in medical errors and adverse events has been the incident reporting system. This method is used to identify high-risk areas that may require and be amendable to structural changes in the healthcare organization. Reporting systems are mainly characterized by a centralized data collection and expert analysis of reports of errors, near-misses, and adverse events by healthcare

professionals; they are confidential or anonymous, non-punitive reporting and provide important information about the type, etiology, outcome and preventability of incidents, suggesting specific interventions to enhance PS. A number of reporting systems with different characteristics are available, but none is totally suitable for all types of errors and adverse events and none is accepted by healthcare professionals in all cases. Mandatory and voluntary reporting systems are the main proposed typologies of incident reporting. The mandatory system focuses on errors resulting in severe harms or death. This system is able to identify only a part of the errors and underestimates iatrogenic ones. The voluntary system focuses on near-misses, allowing to identify weak points in the systems and to improve patients' safety[45]. This system requires that healthcare providers voluntarily collaborate and trust the system, overcoming some barriers as the fear of punishment by superiors or of legal exposure. Snijders *et al*[26] reported that a voluntary and non-punitive approach is suitable in providing important information about the type, etiology, and outcome of incidents, and to suggest appropriate avoiding strategies when applied in the NICU wards. Another reporting system classification includes the comprehensive national voluntary and the specialty-based systems. The first one provides the reporting of all types of medical errors and adverse events, while the second one is tailored to specific branches of healthcare system and allows to identify patterns of errors that are specific to each specialty. It has been documented that healthcare professionals better accept the specialty-based system that is considered more feasible[46]. A voluntary, anonymous, and specialty-based reporting has been found to be more powerful in improving PS, identifying a broad range of medical errors in the NICU and promoting collaborative learning among different disciplines[46].

Recently, a pilot study has been conducted to test the feasibility and utility of performing a real time safety auditing during routine work in a NICU. This tool was shown to promptly identify a wide error range, and allowed to detect significant safety problems. Clinical staff performed safety audits soon after work rounds, having a prompt feedback regarding team efficiency. Rapid changes in practice and policy were adopted by the health caregivers involved in this study, in order to improve PS[47].

Recently, technology systems have been developed to provide further tools for ME prevention by processing inserted data, offering information and an accurate modality of communication, and alerting caregivers in the case of potential error occurrence. The information technology system that is currently recommended in the hospital setting is the Computerized Physician Order Entry (CPOE). It functions as a firewall to reduce the ME risk[44]. However, the CPOE effectiveness in lowering the rate of preventable NICU MEs has not yet been clearly demonstrated. Nevertheless, the opinions of experts and adult and pediatric data support the CPOE use in the NICU. There are different typologies of CPOE, all characterized by the automatic medication-ordering process. Most of the CPOE systems are integrated with a more or less sophisticated clinical decision support (CDS) that provides warnings or suggestions about drug doses, routes and frequencies of administration. The most advanced models are implemented with other important items, for example a drug-drug interaction analysis. A basic CPOE system only accepts typed orders in a standard format, and guarantees a complete, clear and standardized drug order[48]. In the full CPOE system, a doctor prescribes a medication by CPOE and CDS software, which transmits the information to the software of the pharmacy. The latter keeps track of the movements of a robot able to read the electronic drug order and to prepare a specific drug's unit dose to be given to a particular patient, by a specific route and at a stated time. Then, a barcode label holding all this information is automatically tagged to the unit dose, and subsequently delivered to the patient unit. At the patient's bedside, the nurse scans the barcode applied to the unit dose package, the barcode on his/her identification badge, and that on the patient's wristband. The barcode scanner communicates these data to a computerized system which verifies the correspondence with the medical prescription and indicates that the unit dose of the drug can be administered. In the end, the nurse notifies the system that the drug dose has been administered[31]. In 2009, an Iranian study evaluated the effects of CPOE use in a neonatal ward on reducing MEs concerning two drug classes. The error rate remained constant after the CPOE introduction, while decreased significantly from 53% to 34% after the decision support system was added to the CPOE. Prescription error rate, but not transcription error rate, was modified by the introduction of this system. The most frequently intercepted errors were dose errors, namely over-dose errors[3]. New typologies of MEs have been identified after the introduction of CPOE systems. Physicians were found to be at risk for selecting an unrequested drug from a list of multiple proposed medications[36]. Ignoring warnings was identified to be another important reason for the partial failure of CPOE system. A recent study has documented that physicians ignore the warnings when they are not able to understand the reason of the alert appearing, and accordingly they perceive them as inappropriate. Introducing an explanation of warnings that allows the prescribers to understand the reason for the alert appearance has been suggested as a useful tool for increasing the physicians' compliance with the system suggestions, thus further reducing MEs[3].

Unfortunately, in spite of the potential benefits resulting from the CPOE use in preventing MEs, this electronic prescribing system has not yet been introduced into the majority of hospitals for different reasons, including organizational barriers, resistance to change from neonatologists, and high costs.

CONCLUSION

Since 1999, the problem of PS has drawn particular attention, becoming a priority in health care. All healthcare

professionals are susceptible to committing MEs, especially those working in neonatal wards. Newborn infants show an increased risk of MEs because of multiple factors, including small size and reduced compensatory abilities of the neonatal population, the frequent use of unlicensed or off-label drugs in this population, and the complexity of the NICU environment[49].

Although a high ME rate has been found in neonatal wards, newborn safety issues have not been adequately studied until now. Vigilance, training, and dedication are not enough to prevent this type of error, especially in a complex system such as the NICU. Current prevention strategies have been borrowed from the adult wards, but many factors such as high costs and organizational barriers have hindered their diffusion. In the near future, prevention strategies for MEs need to be improved and tailored to the special neonatal population and the NICU environment and, at the same time, every effort will have to be made to support their clinical application. Finally, it is of utmost importance to create a culture of safety among healthcare professionals, with the ultimate aim of improving the general reliability of any system that provides neonatal care.

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REVIEW

Childhood epilepsy and sleep

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Abstract

Sleep and epilepsy are two well recognized conditions that interact with each other in a complex bi-directional way. Some types of epilepsies have increased activity during sleep disturbing it; while sleep deprivation aggravates epilepsy due to decreased seizure threshold. Epilepsy can deteriorate the sleep-related disorders and at the same time; the parasomnias can worsen the epilepsy. The secretion of sleep-related hormones can also be affected by the occurrence of seizures and supplementation of epileptic patients with some of these sleep-related hormones may have a beneficial role in controlling epilepsy.

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Key words: Nocturnal frontal lobe epilepsy; Sleep; Parasomnias; Sleep-related hormones

Core tip: The relation of sleep and epilepsy was discovered many centuries ago. Some types of epilepsies have increased activity during sleep disturbing it; while sleep deprivation aggravates epilepsy. Both are integrating together; so that controlling epilepsy will improve the sleeping quality while consolidating sleep will ease controlling the seizures. Meticulous care of sleep pattern and quality in epileptic children has significant effects for diagnosis, efficacy of controlling seizure activity, and health-related quality of life. Adequate studying of the sleep related disorders and hormones may give a clue for new methods of better epilepsy control.

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INTRODUCTION

Epilepsy is a phenomenon of recurring seizures. A seizure is a result of an increased electrical activity in the brain which causes sudden change in the behavior of the affected person. One third of epileptic patients have seizures during the sleep. On the other hand; sleep disorders are more prevalent in epileptic children and they are common associated epilepsy co-morbidities[1]. This is due to the strong link between the physiology of sleep state and the principal pathological mechanisms of the epileptic seizures[2]. The possible association of epilepsy with sleep was known since long time. Description of episodes of epileptic seizures occurring during sleep was found in the extant writings of both Aristotle and Hippocrates. However, this interactive double-way effect shared between sleep and epilepsy was revealed only by the end of the 19th century by Gower who was interested by the effect of sleep/awakens cycle on generalized tonic-clonic epilepsy[3].

Over the last twenty years, there has been a vast growth in the awareness about the inherent relationship between sleep and epilepsy especially with the wide use of polysomnography and video electroencephalogram (EEG) monitoring. This complex relationship and interaction between sleep and epilepsy was found to be interrelated. This means that epilepsy disturbs sleep and sleep deprivation may aggravate epilepsy due to the decrease in seizure threshold thus forming a vicious circle[4]. About 45% of patients with medically refractory epilepsy experienced excess daytime sleepiness. However, presence of sleep fragmentation without a history of seizures or

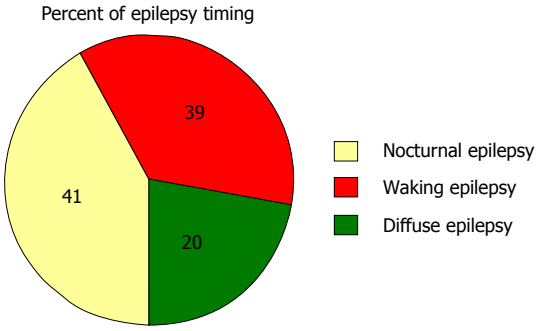

Figure 1 The ratio of the different epilepsy timing.

antiepileptic medication is suggesting that sleep disintegrity may be an intrinsic component of certain types of epilepsy[5].

EFFECT OF SLEEP ON EPILEPSY

Epileptic electrical activities may appear only during sleep

There is a considerable difference in the amount of baseline brain rhythmicity between the states of sleep and wakefulness which could explain that different seizure varieties begin specially in certain sleep states[6]. Almost two-thirds of seizures happen between 8:00 PM and 8:00 AM and about 20% of seizures occurred during sleep (nocturnal epilepsy), 41% occurs during the day (waking epilepsy) and the rest occur during day or night (Figure 1).

Nocturnal seizures most often occur in the early morning near the end of the sleep period (5 AM to 6 AM) and less frequently 1 to 2 h after sleep onset, while diurnal seizures frequently occur in the early morning and late afternoon[7]. Pavlova *et al*[8] showed that frontal lobe seizures occurred more frequently between 12 midnight and 12 midday while temporal lobe seizures, occurred more often between 12 midday and 12 midnight.

Sleep consists of repetitive cycles each lasting about 90 min, advancing through non-rapid eye movement (NREM) stages to rapid eye movement (REM). These two neurophysiological conditions that distinguish sleep (NREM and REM) exert contradictory effects on interictal state and clinical conditions. Many researches showed occurrence of generalized discharges and clinical seizures mainly in NREM sleep. This is the reason that NREM may be considered as a natural "epileptogenic agent"[9]. NREM sleep can enhance interictal epileptiform discharges in both partial and generalized seizures while REM sleep limits spread of epileptic discharges outside the area that started seizure activity and allows the localization of the primary epileptogenic area as seen in temporal lobe seizures. The seizure activating role of NREM sleep has been attributed to increased neuronal synchronisation within thalamo-cortical projection neurons with robust activation of epileptic ictal and interictal activity. Most sleep-related seizures start during sleep stage Ⅱ. Meanwhile, few seizures occur during stages of slow wave sleep (SWS) (stages Ⅲ/Ⅳ) and fewer or none occur during stage of REM sleep. Seizures originating from frontal lobe start during sleep more often than that arise from temporal lobe, a finding that is of clinical significance[5,10-12].

The seizures epileptic activities increased during sleep were illustrated in a number of epileptic conditions and distinguished by uncommon clinical seizures but with significant cognitive impairment. The syndrome of continuous spike-wave discharges during sleep (CSWS) is a typical example of such epileptic conditions. Other sleep augmented epilepsies include benign focal epilepsy of childhood with centro-temporal spikes, Lennox Gastaut syndrome and frontal lobe epilepsies (either supplementary sensorimotor area (SSMA) or autosomal dominant nocturnal frontal lobe epilepsies). Other epilepsies have a tendency to occur upon awakening like Juvenile myoclonic epilepsy; petite male epilepsy; and epilepsy with grand mal seizures on awakening[5]. There are other neurological disorders seen in children that are associated with sleep-activated EEGs. These disorders include Landau-Kleffner syndrome (LKS); pervasive developmental disorder with regression; childhood disintegrative disorder; congenital aphasia (developmental language disorder); and transient cognitive impairment[13].

The epileptic condition termed as CSWS describes clinical epileptic syndromes seen within a condition called electrical status epilepticus in sleep (ESES). In this condition, there is a characteristic electroencephalographic pattern with significant activation and increase in epileptiform discharges during sleep. Some cases of LKS may present only with electrographic seizures, together with the unique ESES which could represent some overlap between CSWS and LKS. However, children with the condition of CSWS who have more global regression will suffer from more challenging epilepsy and the focus of the electric epileptic activity located frequently in fronto-temporal or frontocentral areas. On the contrary, children with LKS develop an acquired aural agnosia, less seizures, and their foci of electric epileptic activity located in the posterotemporal areas. Occurrence of ESES needs a high level of clinical attention and suspicion because slow-wave sleep should be documented and recorded to confirm this condition. Severity of ESES can differ over time in the same patient or differ from patient to another and clinical condition does not necessarily show direct correlation with spike wave index in all the times. However, the prognosis of LKS is significantly superior to CSWS. Treatment of ESES is not only by controlling the seizures; but refinement and improvement of the continuous epileptiform discharge must be done to get good neuropsychological outcome[14,15].

Benign childhood epilepsy with centrotemporal spikes (BECTS) is a type of focal idiopathic epilepsy that is more common in childhood and was previously known as Rolandic epilepsy. It comprises three quarters of benign childhood partial epilepsies, and is characterised by striking ictal clinical manifestations and EEG abnormalities. The typical clinical symptoms include hemifacial convulsions which tend to generalize in sleep. The interictal

EEG shows normal base activity and epileptiform activity, characterized by high amplitude spikes mainly in the central or mid-temporal regions (centrotemporal spikes or rolandic spikes) which are exaggerated by drowsiness or sleep. There is no intellectual defect or cerebral lesion with good prognosis. The seizures usually remit by the age of 15 years[16]. The Sleep style is not notably impaired in BECTS; however; cyclic alternating pattern (CAP) studies show a decrease of NREM instability, mainly in sleep stage Ⅱ. This is most likely related to disturbance of the physiological synchronization mechanisms (needed for the generation of slow-wave components of CAP) by the centro-temporal spikes[17].

Frontal lobe epilepsy is another commonly occurring type of partial epilepsy which originates from the frontal lobe of brain. These seizures show a tendency to arise preferentially during sleep. This kind of epilepsy typically occurs in a cluster of short fits with a rapid start on and off. Some of the common symptoms of this disorder include sudden battering movements during sleep, with the head jerks to one side, and the upper limbs rise with it into a brief, frozen state[5]. Behavioral automatisms are common and include rocking, bicycle pedaling movements and repetitive hand movements. There are two unique main clinical types of epilepsy that originate from the frontal lobe: the first one is that originates from SSMA, and the second one is nocturnal frontal lobe epilepsy (NFLE). BECTS is considered by some authors as a type of frontal lobe epilepsy[18]. The SSMA epilepsy describes the epilepsy that originates from the SSMA located at the mesial aspect of the superior frontal gyrus (area 6) and extends to the dorsal aspect of the lobe convexity. The seizure activities occur principally during sleep. Seizures arising from SSMA characterized by being of brief duration (10-40 s) with occurrence of quick onset of asymmetric tonic posture affecting both extremities. Consciousness is often preserved despite that speech arrest and vocalization are common[13]. These seizures also tend to occur in groups and can be badly disabling. Interictal epileptic sharp waves are frequently found at the midline, with maximum activity at the vertex or just close to the midline in the fronto-central area. The EEG pattern during the fit is characterized by occurrence of a high amplitude transient slow or sharp wave at the vertex, followed by low amplitude fast activity or an electrodecremental style[19].

Autosomal dominant type of NFLE is a rare inherited form of epilepsy that occurs in families. Its onset can start in infants or in adults. It is clinically characterized by occurrence of groups of nocturnal motor seizures, which are usually repetitive in the same manner and of short duration (5 s to 5 min). The seizure may take the form of simple arousals from sleep or as surprising, dramatic and unusual attacks of hyperkinetic fits with tonic or dystonic features. Aura may occur in some individuals with this type. It is frequent to have maintained consciousness during the attacks. A few patients may have fits during the daytime[20]. EEG monitoring during the epileptic fits may be normal or may be fogged up by movement artifact. If present, the characteristic rhythmic pattern of the fits usually takes the form of sharp waves or rhythmic spikes with a rate of 8-11 Hz. There are some data suggesting that these seizures may be initiated with K-complexes[21].

Epileptic electrical activities affected by sleep

Presence of interictal electrical spike activity is one of the characteristic features of epilepsy[22]. Conventionally, sleep-deprivation induces sleep that can be used to trigger interictal electrical spike activity on scalp EEG or magnetoencephalography during evaluation of patients who will be subjected to epilepsy surgery[23]. Earlier researches proved the preferential occurrence of some seizures types during sleep and demonstrated the link between sleep and triggering of epileptic activity on EEG[24-26]. Seizures can be triggered by the synchronized NREM sleep while can be suppressed by desynchronized REM sleep. The association between epileptiform activity and NREM sleep is obviously revealed in the syndrome of continuous spikes in slow-wave sleep and the LKS. The sleep EEG is a helpful diagnostic tool of epilepsy. It also useful to localize the epileptic foci, as new epileptic foci can appear in sleep. REM sleep may aid to reveal the narrowest localization of the primary focus[5].

Previous researches also showed that children with different types of epilepsies (including focal epilepsy, LKS, and CSWS) had greater frequency and topographic field of interictal spike activity during sleep spindle than during wakefulness[27,28]. Earlier studies revealed presence of CAP between the excitatory A phase with K-complex and slow-waves during sleep (which could increase the frequency of interictal epileptic discharges) and the inhibitory B phase with low-voltage fast waves, together with sleep spindles alone[24,29]. However, Asano *et al*[30] showed that sleep could affect the overall frequency and severity of interictal spike frequency but not its topographic distribution. They proved that the topography of spike frequency was rather comparable between wakefulness and sleep with spindles in children who suffered from focal epilepsy. They assumed that sleep with spindles may lower the threshold of triggering the diffuse rather than focal activity of the interictal spike. They found also that both antiepileptic medication and postictal state may affect interictal spike frequency as the post-ictal period was associated with increased frequency of the interictal spike frequency but not simply after tapering antiepileptic medication[31].

EFFECT OF EPILEPSY ON SLEEP

Aadequate, sufficient and good healthy quality of sleep is an integral and important but unfortunately frequently missed component of general health. It is predominantly vital to the proper management of epileptic patients. Ineffective or inadequate sleep is common in epilepsy patients. Their sleep could be disturbed by natures of their seizures, presence of coexisting sleep disorders and

sometimes by seizure medications. The epilepsy may affect all the sleep parameters including night time difficulties, and parent/child interaction during the night. It also can induce sleep fragmentation, parasomnias, and daytime drowsiness. This can result in memory dysfunction; and considerable impairment of daytime functioning, and quality of life. The patients may experience daytime tiredness and impaired attention and arousal for years without knowing the reasons[6]. de Weerd *et al*[32] showed that the prevalence of sleep disturbance was twice more common in patients with partial epilepsy compared with controls and most parameters of sleep were extensively troubled. They had already significant impairment of the quality of life, and which is further compounded by sleep disturbances. Stores *et al*[33] assessed the sleep disturbances in 79 school children with epilepsy by parental questionnaires. They found higher incidence of sleep disorders in epileptic children (mainly poor quality sleep as well as anxieties about sleep) compared with normal controls. They also found associations between disturbed daytime behavior and sleep problems, mostly poor sleep quality among epileptic children aged 5-11 years. The more the frequency of the seizure is; the more the anxieties about sleeping are.

Children suffering from epilepsy were scoring higher for poor sleep quality, about sleep anxiety, and breathing disorders than controls. Cortesi *et al*[34] evaluated 89 children with idiopathic epilepsy for comparisons with 49 siblings and 321 healthy control children using parental questionnaire to assess sleep problems. Their findings indicated that attention to behavioral problems and sleep was important in clinical management of children with idiopathic epilepsy. When seizures occur during sleep, the seizures may awake the patients from sleep which could be mistaken with insomnia. Epileptic patients are frequently not aware of the seizures that occur during sleep. Epileptic children had more problematic sleep disorders than did the controls. These sleep disorders were correlated with how much seizure were frequent, the age of the child, duration of illness, presence of paroxysmal activity on EEG, and behavioral problems. Disturbance of the sleep architectures have been recorded in epileptic patients. This disturbance took different forms. It may appear as interruption and unsteadiness of the protective REM phase of sleep with shortening of REM phase duration. The child also may complain taking long time to go to sleep or from increase frequency of waking up after sleep onset and increased number of arousals, awakenings, and stage shifts[35]. In addition, the type of epilepsy may have a role in sleep disturbances. For example; children with partial epilepsy with secondary generalization or idiopathic epilepsies may suffer more problems and less tolerance to sleep fragmentation than observed in children with generalized tonic-clonic epilepsy or those with symptomatic/cryptogenic seizures. There was a strong association between nocturnal EEG abnormalities (*e.g.*, paroxysmal activity) and the frequency and severity of sleep disturbance which give us a clue that the epileptiform discharges could have an important role in arousal disorders of sleep[34].

The sleep disturbances occurring in epileptic children may be in part due to the effect of drugs used to treat epilepsy. However, the anti-epileptics do not share the same effects on the sleep. Some drugs may have damaging effects on the sleep, predominantly benzodiazepines and barbiturates and others has less damaging effects like phenytoin and, possibly, carbamazepine (CBZ). On the other hand; other drugs may improve the sleep quality especially gabapentin. CBZ is well known to increase SWS[36]. Polytherapy may aggravate sleep related awakening due to parasomnia which may affect both intrinsic and extrinsic features of sleep. Children on more than one antiepileptic drug suffered worse sleep habits than children on one antiepileptic drug[37]. Ketogenic diet treatment despite decreasing the sleep time but it is able to improve sleep quality in epileptic children having drug-resistant epilepsy[38]. Table 1 showed the effects of anteepileptics on sleep. Epilepsy is rarely a syndrome purely of seizures-rather, it is usually accompanied by other cognitive, behavioral, and emotional changes. Epilepsy-associated co-morbidities and other health troubles may also affect sleep. Epileptic children are more risky to develop depression, anxiety, migraine headaches, and obesity which in turn may affect sleep pattern[39].

Considerable sleep impairment in epileptic children could negatively affect their quality of life and impair their seizure control. So, it is of utmost importance to improve all aspects of sleep and should be taken into consideration when treating an epileptic child as sleep can be affected by frequency of seizure occurrence, their location and the balance between the benefits and side effects of antiepileptic used. The concerned treating physician should proactively evaluate sleep and treat any sleep disturbances as part of the comprehensive care of epileptic patients[6]. Many researchers showed marked improvement in sleep quality by proper controlling of nocturnal seizures which improved sleep efficiency, decreased arousals, and associated with increases in duration of REM sleep[40].

EPILEPSY AND PARASOMNIA

Parasomnia is a category of sleep disorders which involve abnormal and unusual behaviours, perceptions, movements, feelings, or dreams that could occur in any sleep phase while going to sleep, sleeping, between individual sleep stages, or during the stage of arousal from sleep. Parasomnias were classified into two main categories of parasomnias according to the sleep state of origin. The first category occurs during stage 3 (or 4) of NREM sleep and called NREM parasomnias or SWS. The second category is REM parasomnias which occur between wakefulness and REM sleep. The NREM parasomnias are further subdivided into disorders of arousal and disorders of sleep-wake transition and include confusional arousals; sleep walking (somnambulism), sleep talking

Table 1 Effects of anti-epileptic drugs on sleep

Antiepileptic	Effect on sleep
Benzodiazepines and barbiturates[70,71]	Reduce sleep latency Decrease the amount of REM sleep Benzodiazepines reduce slow wave sleep Increase incidence of OSA
Phenytoin[70,72,73]	Increases light sleep Decreases sleep efficiency Most studies show decreased REM sleep
Ethosuximide	May cause sleep disturbances, and night terrors
Carbamazepine[72,74,75]	Reduction in REM sleep particularly with acute treatment Effective in treatment of restless legs syndrome sleep disorder
Valproate[73]	May increase stage 1 sleep Could worsen OSA through weight gain
Lamotrigine[75,76]	May cause decreases in slow wave sleep May cause insomnia and sleep disturbance
Gabapentin, pregabalin, and tiagabine[76-81]	Effective in treatment of restless legs syndrome Enhance slow wave sleep and sleep continuity Gabapentin is effective in treatment of restless legs syndrome Pregabalin may cause insomnia and abnormal dreams
Levetiracetam[79,80]	Has little effect or an increase in sleep continuity and slow wave sleep
Zonisamide, rufinamide, oxcarbazepine, and topiramate[82,83]	Have no known effects on sleep and sleep disorders May cause insomnia

REM: Rapid eye movement; OSA: Obstructive sleep apnea.

(Somniloquy); bruxism (teeth grinding), night terrors, restless legs syndrome; and periodic limb movement syndrome. On the other hand; REM associated parasomnias include behaviour disorders of REM sleep, night mares and catathrenia. Catathrenia consists of breath holding and expiratory groaning during sleep, is different from both sleep talking and obstructive sleep apnea (OSA). Because of the higher frequency of arousal disorders, nightmares and bruxism in families with frontal lobe epilepsy; Bisulli *et al*[40] suggested an intrinsic link between parasomnias and NFLE and an abnormal (possibly cholinergic) arousal system as a common pathophysiologic mechanism. Despite being benign disorder, frequent or unusual episodes of parasomnias may occasionally be confused with epilepsy, predominantly NFLE[41]. In cases of diagnostic dilemma video-EEG monitoring is required. Even this, however, may not result in a proper diagnosis, as interictal and ictal EEG findings are frequently unremarkable or nonspecific in both parasomnias and NFLE[42].

EPILEPSY AND OSA

OSA is a common problem in epileptic patients. It presents in about 10% of unselected adult epilepsy patients, 20% of children with epilepsy and up to 30% of drug-resistant epilepsy patients. OSAs results in sleep disruption and deprivation which worsens seizure control and induces drug resistance. It causes repeated brief cessation of breathing during night, sometimes leads to cerebral hypoxemia that could trigger seizure activity. Many researches propose increased incidence of nocturnal seizures during the lighter stages of sleep. OSA is associated with greater incidence of transitory stages of light sleep due to sleep fragmentation which act as a seizure-provocative factor. On the other side, OSA can occur as a side effect of certain epilepsy therapies. For example; vagus nerve stimulation which is used to control the seizures in children with refractory epilepsy above the age of 12 years may enhance OSA in patients with preexisting OSA[43,44]. Barbiturates and benzodiazepines can cause respiratory depression, smooth muscle relaxation and hypotonia of upper airway muscles making them more collapsible. Other antiepileptics (such as valproate, gabapentin, CBZ, pregabalin, and vigabatrin) can induce weight gain as an adverse side effect and hence can promote OSA[45]. Malow *et al*[46] studied the effect of treating the co morbid OSA with medically refractory epilepsy in 35 adult patients using continuous positive airway pressure (CPAP). They found that proper managements of OSA in epileptic patients notably helps in seizure control.

EPILEPSY AND SLEEP-RELATED HORMONES

Many physiological hormonal changes in neuroendocrine system occur in the epileptic patients especially that encountered in the sex hormones. The mechanisms of these neuroendocrine changes are not yet fully elaborated. The hypothalamo-pituitary-adrenal axis function could be impaired acutely during the seizure or may be affected chronically on the long run in epileptic patients. Melatonin is a powerful chronobiotic secreted from the pineal gland; helps to maintain normal circadian rhythms and is used to treat sleep disorders. Bazil *et al*[47] found that patients with intractable epilepsy have low baseline melatonin levels that increase dramatically following seizures. Night-time melatonin intake proved to be helpful in alleviating epileptic activity in children specially

myoclonic types and nocturnal epilepsies. However; it is unclear whether this alleviating effect was through improving quality of sleep or through more specific neuroprotective function[48,49]. On the other hand, some studies reported that melatonin can decrease seizure threshold, and hence could increase seizure activity[50,51]. Gupta *et al*[52] showed that daily doses of 3-9 mg of melatonin could have a valuable effect through its effects on antioxidant enzyme levels and through improving the quality of life in epileptic children.

Some studies demonstrated increase of certain hormones immediately after the seizure. The serum levels of prolactin (PRL) and sex-related hormones (as luteinizing hormone, and follicle-stimulating hormone) were found to increase in the postictal stage in epileptic patients with either generalized tonic-clonic or partial seizures[53]. PRL shows more consistent postictal changes than other hormones. Serum PRL showed transient increase following epileptic seizures. The main reason for such increase in postictal PRL level is probably due to involvement of the temporal lobes and limbic system. This increase could be used to discriminate between true epileptic and psychogenic fits. It is advisable to measure serum PRL with in 10 to 20 min after the fit and is probably a useful measure to differentiate between a grand mal epilepsy seizure, complex partial seizure or psychogenic non-epileptic seizures. In contrast, if the serum PRL level is measured after 6 h from the seizure, then it may indicate the baseline PRL level of the patient. Nonetheless, serum PRL assay cannot differentiate between true epileptic seizures and loss of consciousness due to syncope. At the same time, normal levels of PRL cannot exclude epilepsy or confirm presence of psychogenic seizures because of low PRL assay sensitivity[54,55]. Meanwhile, there are a considerable numbers of studies with conflicting results about PRL increase in epilepsy[56,57].

Despite some hormonal changes other than PRL were observed during the postictal stage, there was no strong evidence of their usefulness for postictal hormonal testing[58,59]. PRL releasing neuropeptide (PrRP) is a peptide with strong PRL-releasing effect from the anterior pituitary cells. Its receptors present not only in the hypothalamic-pituitary axis but also present outside it suggesting that it could have other roles. Lin *et al*[60] found that PrRP has the ability to modify the function of the reticular thalamic nucleus and triggering of non-convulsive absence seizures. These finding open the door for new therapeutic methods able to alleviate sleep disorders and absence seizures. Zhang *et al*[61] showed that adrenocorticotropic hormone (ACTH) and cortisol serum levels showed significant fluctuations in epileptic patients: decreasing below the usual sleep-level shortly before epileptic seizures, rising during epileptic seizures and far above the average wake-level after epileptic seizures. In contrast; Gallagher[62] showed that patients with temporal lobe epilepsy secrete ACTH at higher rates and in greater amounts than normal subjects.

El-Khayat *et al*[63] found that the post provocation growth hormone (GH) levels as well as that of insulin-like growth factor 1 were significantly lower in children and adolescents with idiopathic epilepsy than in the control. This can explain the impaired physical growth pattern observed in the affected children. On the other hand, status epilepticus has a paradoxical GH effect. Lindbom *et al*[64] showed that regulation of GH was significantly altered as a result of the long-standing epileptic activity encountered in status epileptics. Ghrelin is a peptide hormone, which affects both endocrine function and sleep. Berilgen *et al*[65] showed that the mean serum ghrelin level was significantly higher in epileptic patients than the control. This increase in serum ghrelin level might contribute in prolongation of NREM sleep in those epileptic patients, thus may participate in seizure provocation. On the other hand, increase serum ghrelin levels could induce physiological changes in the hormonal secretions or function through its impacts on GH, and in that way play a supplementary role in seizure provocation.

Hormonal changes that occur during puberty could enhance the appearance or remission of certain types of epilepsy. A good example of this hormonal effect is juvenile myoclonic epilepsy which typically develops around the onset of puberty. On the other hand, the childhood absence seizures often remit during puberty[66].

SLEEP HYGIENE IN EPILEPTIC CHILDREN

Adequate sleep helps the overall health and helps to prevent seizures, and improve memory, learning and concentration. Sleep disruption can cause increasing sleepiness during daytime, deterioration of the seizures control, and poor life quality. Proper sleep disorders screening in epileptic children and appropriate interventions will help to improve the quality of life and adequate seizure control[67].

Epileptic patients are in a real need for proper sleep hygiene practice which is more reasonable in epileptic patients than in normal subjects. The need for adequate sleep hygiene practice in epileptic patient appeared as a result of their habitual prevention from exercising many of the activities that could trigger seizure activity or worsen the course of epilepsy or its complications[68].

For a better sleep hygiene, the child is better to have fixed sleeping and awakening times every day including weekends. Naps are better to be avoided if possible. The epileptic patient should be encouraged to have a regular exercise, particularly aerobic exercise, is good not only for the sleep or epilepsy control but also for overall health. However, the child should avoid stimulating or strenuous exercise especially in the evening times (should be avoided no less than 5 h before bedtime).

The child should avoid exhausting, exciting, annoying, or anxiety provoking activities at the bed time, in bed or in the bedroom (watching movies, learning, revising the checkbook, *etc.*). Quiet, relaxing activities and breathing techniques could help the child to feel asleep. Stimulating drinks like cola, coffee, tea, or other caffeinated beverages should not be taken after about noon. He should

not have chocolate by the evening time. The child should keep away from drinking many liquids in the evening to avoid disturbing the sleep by needing the toilet. Sedating anti-epileptics should be lessened if possible during the day, and activating anti-epileptics should be used when suitable. Weight gain should be avoided in patients with sleep apnea. Obese patients with sleep apnea should avoid using anti-epileptics that may promote weight gain; instead they should be encouraged to use anti-epileptics that cause weight loss. CPAP is still the treatment of choice for sleep apnea[69].

CONCLUSION

Epilepsy and sleep are integrating together; controlling epilepsy will improve the sleep quality while consolidating sleep will make it easier to control the seizures. Meticulous care of sleep habits in epileptic children has important effects in epilepsy diagnosis and control as well as quality of life. Adequate studying of the sleep related disorders and hormones may give a clue for new methods of better epilepsy control.

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MINIREVIEWS

Cyclical vomiting syndrome: Recognition, assessment and management

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Abstract

Cyclical vomiting syndrome (CVS) is a functional, debilitating disorder of childhood frequently leading to hospitalization. Affected children usually experience a stereotypical pattern of vomiting though it may vary between different individuals. The vomiting is intense often bilious, and accompanied by disabling nausea. Identifiable precipitating factors for CVS include psychosocial stressors, infections, lack of sleep and occasionally even food triggers. Often, it may be difficult to distinguish episodes of CVS from other causes of acute abdomen and altered consciousness. Thus, the diagnosis of CVS remains largely one of exclusion. Investigations routinely done during the work-up of a child with suspected CVS include both blood and imaging modalities. Plasma lactate, ammonia, amino acid and acylcarnitine profiles as well as urine organic acid profile are indicated to exclude inborn errors of metabolism. The treatment remains challenging and targeted at prevention or shortening of the attacks and can be considered as abortive, supportive and prophylactic. Use of non-pharmacological therapy is also part of the management of CVS. The prognosis of CVS is variable. More insight into the pathogenesis of this disorder as well as role of non-pharmacological therapy is needed.

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Key words: Cyclical vomiting syndrome; Childhood; Pathogenesis; Investigations; Treatment

Core tip: Cyclical vomiting syndrome (CVS) is a functional childhood disorder which has been increasingly reported in recent years. Much of its pathogenesis remains unknown. Diagnosis may be delayed if not considered by paediatricians. Management of CVS still remains a challenge.

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INTRODUCTION

Cyclical vomiting syndrome (CVS) is a functional disorder in childhood which is increasingly being recognised. It is characterised by discrete episodes of recurrent profuse vomiting which are self-limiting and with periods of well-being between the attacks. Each episode is often stereotypical for the individual, in terms of onset, duration and symptoms but may vary between different individuals. The vomiting can be profuse, with bilious content occurring in most cases, and associated with extreme nausea and lethargy. Each attack can be debilitating as the child often spends days being hospitalised for intravenous hydration. Several guidelines have been written in recent years to clarify the diagnostic process of CVS and rule out other conditions that may have similar presentation.

Table 1 North American Society for Pediatric Gastroenterology, Hepatology and Nutrition criteria for cyclical vomiting syndrome

All of the following criteria must be met to fulfil the definition of CVS
At least 5 attacks in any interval, or
A minimum of 3 attacks during a 6-mo period
Episodic attacks of intense nausea and vomiting lasting 1 h 10 d and occurring at least 1 wk apart
Stereotypical pattern and symptoms in the individual patient
Vomiting during attacks occurs at least 4 times/h for at least 1 h
Return to baseline health between episodes
Not attributed to another disorder

CVS: Cyclical vomiting syndrome.

EPIDEMIOLOGY/PATHOGENIC BACKGROUND

The pathogenesis of CVS remains unknown but there appears to be a link between CVS and migraine, suggestive of a central aetiology. There are similarities in the symptoms for both conditions (*e.g.*, nausea, photophobia, and headache) as well as some similarities in triggering factors (*e.g.*, stress, lack of sleep). Interestingly, there is often co-existing personal or family history of migraine in individuals with CVS[1-3]. One possible explanation is that of maternal inheritance of CVS and mitochondrial DNA mutations causing deficits in cellular energy production[4]. Personal history of motion sickness has also been seen in cases of CVS.

In recent years, there have been increasing reports of cases of CVS worldwide. However, the true prevalence and incidence of CVS is difficult to establish as it often remains undiagnosed after the onset of symptoms.

Population based studies in school aged children done in Western Australia[5] and Scotland[6] showed prevalence rates of 2.3% and 1.9%, respectively. Similarly, Ertekin *et al*[7] reports a 1.9% prevalence rate in a cohort of school aged Turkish children. Reported incidence rates range from 1.7% to 2.7% of school aged children[6,8]. In Ireland, an incidence of 3.15 per 100000 children per annum was reported in 2005[9].

CLINICAL FEATURES

The classic patient with CVS is female (slight female predominance with female to male ratio of 1.3:1)[10], who presents with vomiting in childhood. Affected children usually experience a stereotypical pattern of vomiting classically with a consistent time of onset, duration and symptoms. The vomiting is intense (median 6 times/h at peak), often bilious (83% in some series), and accompanied by disabling nausea[11].

CVS classically has four phases: inter-episodic, prodromal, vomiting, and recovery. Recognition of this phasic pattern helps in making the diagnosis and in management. The inter-episodic phase is usually symptom free. The patient senses the approach of an episode during the prodromal phase, but is still able to take and retain oral medications. The vomiting phase is characterized by severe nausea, vomiting, retching, and other symptoms. The recovery phase begins when nausea remits and ends when the patient has recovered appetite, strength, and body weight lost during the vomiting phase.

Identifiable precipitating factors for CVS include psychosocial stressors, infections, lack of sleep and occasionally even food triggers. The resulting dehydration necessitates intravenous hydration. Often, accompanying symptoms such as pallor, listlessness, anorexia, nausea, retching, abdominal pain, headache, and photophobia may make it difficult to distinguish episodes of CVS from other causes of acute abdomen and altered consciousness.

CVS has been reported to occur in all age groups. The median age at onset of symptoms ranges from 5.2 to 6.9 years[11] although children as young as 6 mo have been described to have CVS.

DIAGNOSIS AND INVESTIGATIONS

Often times, in areas where the diagnosis of CVS is not well-known, many patients may spend months over repeated hospital admissions before a diagnosis is made. Patients are often misdiagnosed as food poisoning, gastro-esophageal reflux disease or peptic ulcer disease.

The diagnosis of CVS remains largely one of exclusion. In a child with recurrent vomiting, it is important to rule out life-threatening conditions such as gastro-intestinal structural anomalies including malrotation with volvulus, brain tumours and inborn errors of metabolism. Children with epilepsy can occasionally present with recurrent vomiting, especially if it involves the occipital lobe. The overall lower frequency of attacks and the higher peak intensity of vomiting in CVS usually allow its distinction from disorders such as bulimia nervosa[12]. While CVS can occur in infants and young children, a diagnosis should be made after a period of observation and careful exclusion of other causes of recurrent vomiting[13].

In 2008, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition appointed a task force to develop a consensus report for CVS to improve the recognition and treatment of this disorder. Table 1 shows the criteria for the diagnosis of CVS based on clinical symptoms[14]. Investigations that are routinely done during the work-up of a child with suspected CVS include both blood and imaging modalities. Figure 1 gives a summarized overview of the investigations that follow in the work-up of CVS.

Blood investigations

Serum electrolytes are often assessed in the acute setting, prior to starting intravenous fluid therapy. This often reveals a high anion gap metabolic acidosis in keeping with on-going ketosis during an acute attack which resolves with intravenous hydration. Persistence of electrolyte abnormalities or metabolic acidosis may suggest an underlying metabolic, endocrine or renal disorder. Plasma lactate,

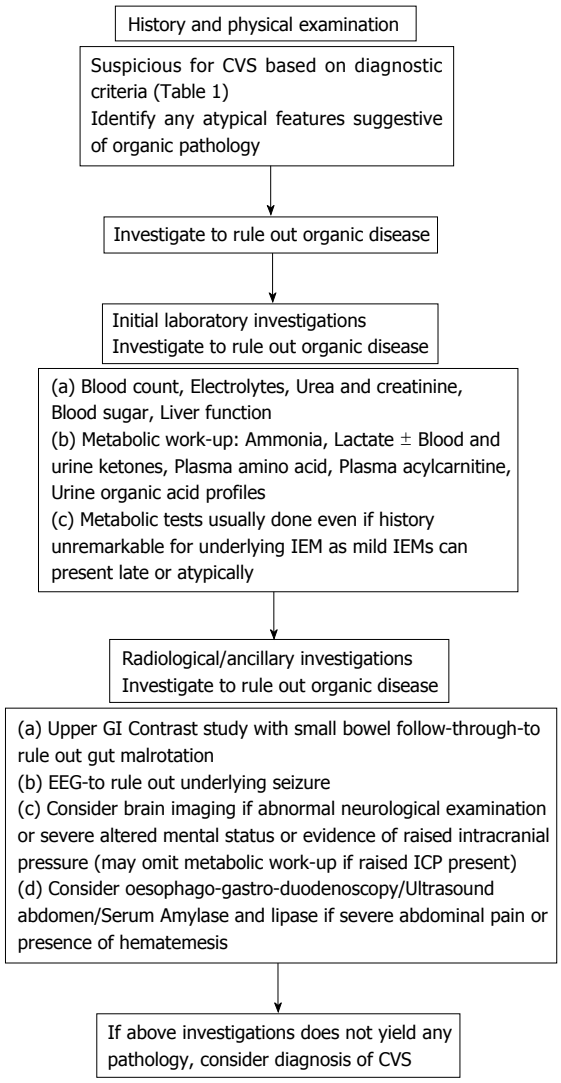

Figure 1 Flow chart for the diagnostic evaluation of cyclical vomiting syndrome. CVS: Cyclical vomiting syndrome; IEM: Inborn errors of metabolism; EEG: Electrical-encephalogram; GI: Gastro-intestinal; ICP: Intracranial pressure.

ammonia, amino acid and acylcarnitine profiles as well as urine organic acid profile are indicated to exclude inborn errors of metabolism (IEM). Children with mild and/or rarer forms of IEM (such as Succinyl-CoA: 3 Ketoacid CoA transferase deficiency) may present later in life and/or with subtle abnormalities such as hypoglycaemia, persistence of metabolic acidosis/ketosis or a clinical picture of encephalopathy when they are unwell, similar to the presentation of CVS.

Imaging modalities

Bilious vomiting requires imaging of the gastrointestinal tract to exclude obstructive causes of vomiting such as gut malrotation. Usual imaging options include an abdominal radiograph, contrast/barium study and ultrasound abdomen. Oesophago-gastro-duodenoscopy is occasionally done in children with symptoms of oesophagitis that persist beyond vomiting episodes.

Brain imaging is often done to rule out tumours of the central nervous system, especially if there is early morning emesis and neurological findings on examination. Although the role of electro-encephalogram in the diagnostic evaluation of CVS remains uncommon, it has been shown that a mild encephalopathic state often exists during an attack and resolves when the child is well. This mode of investigation may lend further support to the diagnostic evaluation of a child with CVS.

TREATMENT

The most controversial and challenging aspects of this disorder are the types of effective treatment, and the duration of medical therapy. No specific therapy has been proven to be effective for CVS in controlled trials. Medical therapy remains the tool to prevent or shorten attacks[15]. Several empiric treatments have been shown to be effective in case series[16]. Treatment can be considered as abortive, supportive and prophylactic.

At the start of an attack, care-givers may try sublingual ondansetron or suppository domperidone to suppress the attack. Sumatriptan has also been reported to be beneficial[17]. Unfortunately in the majority of children, it will not prove possible to abort the episodes of vomiting and many will require a period of hospitalisation.

Acute management

On admission to the hospital, it is essential to assess the severity of dehydration and commence intravenous fluid containing glucose and electrolytes appropriate for the degree of dehydration. Symptom minimisation is necessary not only to make the child feel more at ease but also to alleviate parental distress. Intravenous ondansetron is an effective anti-emetic agent that has been shown to decrease the duration of an episode by more than 50%[11]. Anti-emetic agents used in adults are avoided as they have the associated risk of extra-pyramidal side effects.

The child should ideally be resting in a quiet environment with minimal lighting. Sedation and anxiolytic agents such as chlorpromazine or lorazepam are important as sleep decreases vomiting frequency. Success with alpha blockade using dexmedetomidine[18] and clonidine[19,20] has been reported for small numbers of patients. Proton pump inhibitors or H2 receptor antagonists should be considered for children who have prolonged or frequent episodes of vomiting.

Prophylactic treatment

Limited evidence-based recommendations exist on the use of prophylactic agents in CVS. While patients invariably do well even without long term prophylaxis[21], prophylaxis should be considered in patients who have more than one episode per month, leading to hospitalisations and school absences, and/or with poor response to abortive treatments[14].

Amitryptiline, a tricyclic antidepressant is the first line prophylaxis recommended in children more than 5 years of age[14]. Tricyclics reduce brain-gut and sympathetic autonomic dysfunction by decreasing cholinergic neuro-

transmission and modulation of alpha adrenoreceptors. A systematic review in 2012 showed good response rates (68%-76%) in both adults and children with CVS[22]. In children less than 5 years old, cyproheptadine, an antihistamine and serotonin receptor antagonist, is recommended as the first-line agent. A response rate of 83% was shown in a small series of 6 patients[23].

Propranolol has shown moderate efficacy and is recommended as second line prophylaxis in children of all age groups[14,24]. L-carnitine, a mitochondrial co-factor involved in long-chain fatty acid transport, is also being used as complementary therapy with satisfactory response rates in small case series[25,26].

Various combination therapies have also been tried. Combination treatment using amitriptyline and L-carnitine was found to reduce symptoms in 76.7% of patients[22]. Another combination protocol using coenzyme Q, L-carnitine plus amitriptyline (or cyproheptadine in < 5 years old) has shown > 75% efficacy in episode prophylaxis[27].

Other agents empirically used for prophylaxis with variable success are anticonvulsants valproate and phenobarbital; erythromycin, coenzyme Q, low estrogen oral contraceptives[14,28].

Non-pharmacological

As with migraine treatment, for patients who are able to identify precipitating factors such as lack of sleep, stressful situations or certain foods, prevention of attacks may be possible. Adapting to a low amine diet and avoidance of identified food triggers such as cheese, chocolate or drinks containing caffeine has been described as a prophylactic regimen for CVS with a response rate of 86%[29,30]. A retrospective review by Lee *et al*[21] (2012) also found sleep to be the most common non-medical management (46.2%) in both adult and childhood-onset CVS

Among children with CVS, anticipatory anxiety related to life events such as school examinations, family conflicts have often been identified[31]. Hence, it has been postulated that there is a role for psychological treatment in the management of CVS. Unfortunately, literature on specific psychological treatment for CVS is scarce. Consultation with a psychologist may be beneficial in exploring mental stressors and developing techniques for coping with anticipatory anxiety. A case report by Slutsker explored using cognitive behavioural therapy (CBT) with biofeedback training in a phase oriented manner, targeting on the prevention of symptomatic episodes[32].

CONCLUSION

The prognosis of CVS is variable. Most children outgrow symptoms during adolescence, some trade cyclic vomiting for migraine headache and others continue to have episodes into adulthood[33].

During the interval periods between attacks, it is crucial for the paediatrician or gastroenterologist to review the child. Not only to establish that the child remains well, but also to identify possible psycho-social overlays that may have resulted with recurrent CVS attacks and resultant school absenteeism and poor performance. In children with severe CVS, psychological support may be crucial, not only for the child but also for the distressed parents.

It would be interesting if more insights can be discovered into the pathogenesis of this disorder. This would certainly improve diagnostic and treatment options. At present, treatment options in children remain limited and treatment outcomes often take days before improvement is noted. Further research in the area comparing psychotherapy such as CBT and biofeedback training protocol to both pharmacotherapy and placebo among children with CVS would be a way forward in the management of CVS.

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OBSERVATIONAL STUDY

Recurrent headaches may be caused by cerebral toxoplasmosis

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Abstract

AIM: To establish seroprevalence and provide characteristics of Toxoplasma gondii (TG) infection in children with recurrent headaches.

METHODS: The study was performed in 178 children aged 7-17 years admitted consecutively to the Department of Pediatric Neurology from November 2009 to July 2011. The children were surveyed with a questionnaire with the help and assistance of their parents and blood samples taken on admission were studied for the presence of specific anti-TG IgM, IgG antibodies and IgG avidity using enzyme immunoassay Platelia Toxo IgM, IgG.

RESULTS: The study showed that 19 children (8 boys, 11 girls; 8-17 years old, mean age 14.36 years) had high serum anti-TG IgG antibody levels (range: 32.2 > 240 UI/mL, mean 120.18 UI/mL; positive value for IgG was ⩾ 9 UI/mL). The avidity index (AI) ranged from 0.202 to 0.925 (scale: ⩾ 0.5 high AI). The results for IgM antibodies were all negative and the obtained results ranged from 0.113 to 0.25 U/mL (mean = 0.191 IU/mL) and all values below 0.8 IU/mL were considered negative. The most frequent complaints found in the seropositive patients were headaches that affected the frontal (13 children), occipital (4) and parietal areas (5). Headaches usually had a pulsating (in 7 patients) and squeezing (6) character and rarely were piercing, dull or expanding. Interestingly, 8 children did not feel discomfort during the headaches, probably because they did not have sufficiently increased intracranial pressure yet. The headaches usually appeared 1-2 times/mo, lasted for 2-6 h, and had a mean intensity of 5.5 points in a 10 point subjective scale. The comorbidities included epilepsy (5 patients), various infections in 3 children (chronic eustachitis, chronic rhinitis, chronic purulent tonsillitis, streptococcal pharyngitis, meningitis, allergic diseases), disturbances of behavior, deficits of attention, and ocular and motor concentration disorders in 1 child. The electroencephalographic and neuroimaging studies performed in our patients had a very limited value in establishing cerebral toxoplasmosis.

CONCLUSION: Ten point six seven percent of the studied children had markedly increased serum anti-TG IgG antibodies and high AI indicated chronic infestation. It is suggested that tests for TG infection should be introduced to routine diagnostics in patients with recurrent headaches.

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Key words: Recurrent headaches; Children; Seroprevalence of anti-Toxoplasma gondii IgG antibodies; IgG avidity; Chronic Toxoplasma gondii infection; Cerebral

toxoplasmosis

Core tip: This work estimated the seroprevalence and characteristics of Toxoplasma gondii (TG) infection in 178 children admitted consecutively to the Pediatric Neurology Department because of recurrent headaches. Nineteen children had significantly increased serum anti-TG IgG antibody levels and high avidity index which indicated chronic infection. It is suggested to treat these patients specifically for 5-7 d and eventually be aware of the Jarisch-Herxheimer reaction.

Prandota J, Gryglas A, Fuglewicz A, Żesławska-Faleńczyk A, Ujma-Czapska B, Szenborn L, Mierzwa J. Recurrent headaches may be caused by cerebral toxoplasmosis. *World J Clin Pediatr* 2014; 3(3): 59-68 Available from: URL: http://www.wjgnet.com/2219-2808/full/v3/i3/59.htm DOI: http://dx.doi.org/10.5409/wjcp.v3.i3.59

INTRODUCTION

Migraine and/or other types of headaches are a common concern affecting 5% and 10% of children and adolescents, respectively, as well as nearly 30% of reproductive-age women[1,2]. Studies have shown that children with recurrent headaches are subjected to an ever-increasing risk of such diseases in adulthood as well as many physical symptoms and psychiatric disturbances, such as depression disorders[3]. According to the National Headache Foundation, each year up to 45 million United States citizens suffer from chronic and recurrent headaches with productivity loss amounts each year as much as 50 billion dollars due to work absence and health care costs generated by headache attacks[4]. From 1989-1990, the Australian National Health Survey conducted an analysis of 57000 people, concluding that about 12.2% of them had reported headaches in the past two weeks. As many as 280000 people had typical migraine attacks, while 2 million people reported more subtle forms of headaches during the same 2 wk period[5,6].

Toxoplasma gondii (TG) is a ubiquitous protozoan parasite, infecting up to 30 species of birds and mammals, including the population of humans. Chronic asymptomatic infestation affects 30%-50% of the world population with hosts of parasite cysts located mainly in the central nervous system[7,8]. It is estimated that in continental Europe, around 50%-80% of the entire human population has a latent TG infection. In Paris, 84% of pregnant women had serum antibodies against the parasite[7], while in Poznań (Poland), the number reaches 60%[9]. In Poland, the majority of pigs, cattle and sheep (approximately 80%) have positive serological tests for TG infection[9,10]. It is known that domestic pets may be a source of TG infection because it was reported that, for example, even turtles were susceptible to the RH strain of TG and harbored the parasite for at least a month[7,11,12]. Studies involving immune-competent patients with acquired toxoplasmosis revealed that the most common symptoms of acquired cerebral toxoplasmosis were headaches and peripheral lymphadenopathy (88% and 77% of patients, respectively)[13]. Unfortunately, cerebral toxoplasmosis is not mentioned in textbooks or scientific papers as a cause of various types of headaches. Prandota[14-16] reported that pediatric and adult patients who suffered from headaches severe enough to undergo hospitalization had lumbar punctures and several other diagnostic tests performed but were finally found to have TG infection. The complaints disappeared after repeated anti-parasite treatment regimens. It must be noted that at present patients with migraine and other types of headaches usually receive only symptomatic drugs. The recurrent character of the headaches and frequently applied expensive diagnostic procedures may suggest that children and adolescents suffering from the condition should have serological tests for TG infestation commonly performed.

The purpose of this study was: (1) to estimate the seroprevalence of TG infection in the population of children hospitalized with recurrent headaches and symptoms and/or signs of the central nervous system abnormalities; (2) to establish whether it was an acute or chronic infection; (3) to summarize patients' complaints and describe clinical symptoms with special attention to their duration, intensity, frequency and abnormalities in laboratory data, including electroencephalographic (EEG) and neuroimaging procedures; and (4) to assess associated comorbidities.

MATERIALS AND METHODS

The study was performed in 178 children aged 7-17 admitted consecutively from November 2009 to July 2011 to the Department of Pediatric Neurology in Wroclaw. All of them reported headaches as a major complaint and the reason for hospitalization but also as a secondary condition.

The children were surveyed with a questionnaire with the help and assistance of their parents and blood samples were taken on admission in order to do necessary laboratory tests and study the presence of specific anti-TG IgG, IgM and the index of IgG avidity.

To collect demographic, social and medical data, a specially prepared questionnaire was used. It consisted of three parts: (1) the general part included questions concerning the place of living and contact with domestic pets; (2) the family history with a special regard to occurrence of neurological disturbances, including headaches, in first-blood family members; and (3) the epidemiological part dealing with frequency, intensity and character of headaches, affected part of the head, concomitant symptoms and circumstances correlated with the onset of pain, as well as the degree of discomfort.

After the informed consent of parents was obtained, fasting blood samples were collected from children with headaches. Each sample was centrifuged for 10 min with 3000 rpm and then serum was extracted and kept in

Table 1 Demographic data and positive serum anti-Toxoplasma gondii IgG concentrations in 19 of 178 children hospitalized because of recurrent headaches

Pt	Age/sex	Living place	Contact with animals[1]	Serum anti-Toxo IgG concn ($n \leq 9$ IU/mL)	Avidity index[2]
1	8/M	Town	Yes[1]	205.5	0.750
2	9/M	Town	Yes[1]	59.5	0.754
3	9/F	Town	Cat	59.0	0.202
4	12/F	Town	Cat	94.1	0.666
5	13/F	Town	No	168.0	0.782
6	13/F	Town	Yes[1]	83.0	0.800
7	13/F	Town	No	123.9	0.861
8	14/F	Village	Yes[1]	151.2	0.740
9	15/M	Town	Yes[1]	231.4	0.670
10	16/F	Town	Yes[1]	81.0	0.859
11	16/F	Town	Dog	74.7	0.767
12	16/M	Town	Ferret	165.7	0.778
13	17/M	Village	Yes[1]	175.03	0.788
14	17/F	Town	Yes[1]	145.0	0.772
15	17/F	Town	Yes[1]	40.5	0.687
16	17/M	Town	Yes[1]	> 240.0	0.843
17	17/F	Village	Yes[1]	71.7	0.925
18	17/M	Town	Dog, parrot	32.2	0.796
19	17/M	Village	Dog, cat, hens, ducks, cows	82.0	0.894

[1]Dog, cat and/or turtle. [2]Avidity index: < 0.4-low avidity, 0.4 to 0.5-moderate, and ⩾ 0.5-high avidity. In Pt 13 control serum anti-Toxo IgG concentration examined 9 mo later was 552.7 IU/mL. It must be noted that Toxoplasma gondii IgG and IgA, but not IgM, antibody titers were also found to be increased in sera of immunocompetent mice in association with proliferation of tachyzoites in the brain during the chronic stage of infection[17].

-20 ℃ until assayed. The serum samples were analyzed for the presence of specific anti-TG IgM and IgG antibodies using the enzyme-linked immunosorbent assay (ELISA) technique (Euroimmun) and the index of avidity (Platelia, Toxo™ IgG Avidity). The study was approved by the Bioethics Committee of the Medical School.

RESULTS

Among the studied group of 178 children admitted consecutively to the Department of Pediatric Neurology because of recurrent headaches, sometimes with various other accompanying neurological disturbances, and investigated for the presence of TG infection, serum tests of 19 children (11 girls, 8 boys) detected a marked increase of specific serum anti-TG IgG antibody concentrations (Table 1). The results ranged from 32.2 to over 240 IU/mL, with the mean value of 122.2 IU/mL, and the positive cut-off value for serum anti-TG IgG level was ⩾ 9 IU/mL. The results for IgM antibodies were all negative and the obtained results ranged from 0.113 to 0.25 U/mL (mean = 0.191 IU/mL), all values below 0.8 IU/mL were considered negative. It can be assumed that almost all children with positive IgG tests had chronic TG infection because in 18 children (except Pt 3) the index of avidity ranged between 0.67 and 0.925 and the value AI ⩾ 0.5 was considered high (Table 1).

In 19 children that were TG IgG-seropositive, the most common complaints were headaches reported by all patients. In 13/19 children, headaches affected mostly the frontal region, in 5/19 the temple and in 4/19 the occipital area. The headaches were bilateral in half of the children, while the other part reported headaches of changing, unilateral or bilateral character. The frequency of reoccurrence was about once a month in 7 children, 6 children had more frequent headache attacks, and two of them more rarely (Table 2).

Five out of 19 TG IgG-seropositive patients considered headaches a serious discomfort and 2 children confirmed that they were afraid of pain or anxious about its reoccurrence. Headaches usually were pulsating (in 7 patients) and squeezing (6), and rarely had a piercing, dull or expanding character. Interestingly, 8 children did not feel discomfort during the headaches. The mean duration of headaches was 2-6 h and the average gravity assessed according to 10 grade arbitrary scale was 5.5 (range 2-10) (Table 2). Comorbidities were assessed as well and the most frequent were epilepsy (5 patients), various infections in 3 children (including chronic eustachitis, chronic rhinitis, chronic purulent tonsillitis, streptococcal pharyngitis, meningitis and allergic diseases), disturbances of behavior, deficits of attention, and ocular and motor concentration disorders in one child (Table 3).

The most common disorder detected in accessory diagnostic tests was an abnormal EEG record, observed in 10 out of 15 TG-seropositive patients. It should be noted that 5 of these children were on antiepileptic therapy (Table 4). In 2 patients, X-ray of the paranasal sinuses revealed thickened sinus mucosa probably due to the chronic maxillary sinusitis. On magnetic resonance imaging (MRI), one 17-year-old girl (Pt 15) was suspected of having a cerebellar venous angioma and Kimmerle anomaly, another 17-year-old boy (Pt 17) was monitored concerning the demyelinating process, and 13-year-old girl (Pt 6) had a pia mater cyst, differentiated with atresia of a Turkish saddle diaphragm (Table 4).

DISCUSSION

The results of our study showed that monitoring of anti-

Table 2 Characteristic features of headaches in the seropositive anti-Toxoplasma gondii IgG children

Pt	Frequency of headaches	Pain localization	Duration (h)	Pain intensity[1]	Pain character	Discomfort for patient
1	Once a month	Temples	< 2	6	Squeezing	No
2	Once a month	Forehead	2-6	4	Squeezing	N/A
3	Once a month	Back	< 2	5	Pulsating	No
4	Daily	Whole head	2-6	5	Squeezing	Yes
5	Once a month	Temples, forehead	2-6	5	Squeezing	Yes
6	Once a month	Forehead, occiput	< 2	3	Pulsating, paroxysmal	N/A
7	More than once a month	Forehead	< 2	6	Expanding	Manageable
8	Rarer than once a month	Stomach	< 2	2	Squeezing	No
9	More than once a month	Forehead, occiput	2-6	8	Expanding, piercing	Yes
10	More than once a month	Forehead, temples	2-6	5	Pulsating	Small
11	More than once a month	Forehead (left side)	2-6	10	Expanding	Yes
12	More than once a month	Temples, orbitae (left side)	2-6	7-8	Pulsating, squeezing	Yes
13	Rarer than once a month	Forehead	< 2	5	Pulsating	No
14	Once a month	Forehead, temples	2-6	4	Squeezing, pulsating	Yes
15	Once a month	Forehead, occiput	0.5-24	2	Dull	No
16	More than once a month	Forehead, temples	6-12	5	Pulsating	No
17	N/A	N/A	N/A	N/A	N/A	N/A
18	Once every 3 mo	Forehead, calvaria	< 2	4	Piercing	No
19	Once every 6 mo	Forehead, orbitae	< 2	8	Squeezing	No

[1]Headache intensity was measured in a subjective 1-10 point scale. N/A: Data not available.

Table 3 Concomitant symptoms and comorbidities in the seropositive anti-Toxoplasma gondii IgG children with recurrent headaches

Pt	Concomitant symptoms	Comorbidities
1	Fatigue	Epilepsy, attention and concentration disturbances
2	Autoaggression	Epilepsy, behavior disturbances, psychomotor development delay
3	Vertigo, nausea and vomiting, discrete balance disturbances, photophobia	Infection of upper airways, right eustachitis, suspected allergic rhinitis
4	Nausea, vomiting, abdominal pain, photophobia	Allergic lesions of nasal mucosa and paranasal sinuses, heart systolic murmur (2/6), overweight, aggression and autoaggression behavioral disturbances, attention and concentration deficits, myoclonus during sleep
5	Nausea, vomiting, abdominal pain	Allergy to dust and mites, serous rhinitis
6	Vertigo, changes of mood and behavior, fatigue	Frequent upper airway infections
7	Psychomotor slowdown, episodes of awareness (switch-offs)	Bronchial asthma, paranasal sinusitis, allergic rhinitis, obliteration of right optical nerve disc
8	Impairment of cognitive abilities, psychomotor hyperactivity, clumsy gait	Metachromatic leukodystrophy, significant peripheral nerve lesions-sensorial-motor axonal-demyelinating polyneuropathy
9	Nausea, photophobia, abdominal pain	Bronchial asthma, chronic eustachitis and rhinitis, allergy to dust
10	Photophobia, fatigue, hyperacusis, faintings, syncope	Eye refractive error, serous cerebrospinal meningitis at the age of 6
11	Vertigo, hot flushes, weakness	Traffic accident injury of head and right thigh, diagnosed cerebral commotion, incisive wounds of forehead, nose and over upper lip, positive tetany tests-elevated plasma calcium levels
12	Aphasia, dysphasia, paresthesia of hands and forearms	Bronchial asthma
13		Chronic purulent tonsillitis, streptococcal infection, epilepsy (observation), sclerosis multiplex (observation)
14	Fatigue	Epilepsy
15	Hyperacusis, drowsiness	Epilepsy, attention and concentration disturbances, impaired perception skill, eye-motor concentration disturbances, allergic rhinitis
16	Photophobia	
17	Individual incident of vertigo and limb numbness without loss of consciousness	Vision disorders and ventricular arrhythmia in anamnesis, left-eye divergent strabismus
18	Systemic vertigo, balance disturbances, nausea	Frequent upper airways infections, enlarged submandibular lymph nodes, right palpebral narrowing and discrete right-sided ptosis since early childhood
19	Nausea, vomiting, photophobia, numbness of tongue and right arm	Bronchial asthma, atopic dermatitis, mitral valve insufficiency

TG IgG antibody levels and estimation of the avidity index are an important part of the diagnostic and therapeutic process in children and adolescents with recurrent headaches and associated comorbidities seeking neurological help. Despite the evidence concerning a correlation between acquired toxoplasmosis and increased incidence of neuropsychiatric symptoms, including headaches, vertigo, attention deficits and educational difficulties[14,16,18], there is still lack of broader information regarding acquired toxoplasmosis as one of the impor-

Table 4 Results of laboratory tests and head X-ray/neuroimaging studies in the seropositive anti-Toxoplasma gondii IgG children with recurrent headaches

Pt	ESR (mm/h)	WBC (10^3/mL)	CRP (mg/L)	EEG	Head X-ray/CT/MRI
1		6.4		Increased percent of slow waves	CT normal
2	10	14.9	0.10	Abnormal	CT normal
3		4.0; 6.5	20.25; 1.92	Normal	MRI-brain and fluid spaces normal, massive lesions of paranasal sinuses, total obliteration of the right maxillary sinus, thickened ethmoid mucosa
4		5.4	0.57	Paroxysmal parietal and temporal alterations without paroxysmal activity	MRI-arachnoidal cyst at the right frontal-parietal border
5	22	6.9		Normal	CT normal; X-ray-thickened right sphenoid mucosa
6		6.9		Discrete bilateral alterations in frontal regions	MRI-arachnoidal cyst, underdevelopment of Turkish saddle diaphragm; X-ray-thickened right maxillary sinus mucosa
7		6.5	0.27	Discrete bilateral alterations in temporal regions-increased slow waves	MRI-brain and fluid spaces normal, small and hypoplastic frontal sinuses with thickened mucosa, moderate thickening of ethmoid mucosa and moderate inflammatory or post-inflammatory lesions of maxillary sinuses
8	16	5.7	0.30	Generalized alterations	MRI-extensive lesions (differentiation between metabolic and demyelinating disorder)
9		7.1		Generalized alterations: many dispersed theta waves; biparietal paroxysmal alterations during HV	CT and sinus X-ray normal
10	12	8.4	0.60	Normal	CT normal
11		4.7	0.86	Readout unavailable-pending	Head CT and X-ray of cervical spine normal; MRI-modest lateral ventricle asymmetry, some minute fluid foci adjacent to pineal gland
12		7.5		Normal	MRI normal
13a	8	4.5	0.41	Increased percent of slow waves; increased number of theta waves during HV	MRI demyelinating foci (June 2009); partial regression of these abnormalities (February 2010)
14		6.9	0.39	Slowed down basal function, biparietal alterations	MRI normal
15	24	9.2	1.45	Paroxysmal bioccipital alterations	MRI-venous angioma suspected in right cerebellar hemisphere; X-ray-Kimmerle foramen in C1 of cervical spine
16	9	6.59			CT normal
17		10.6		Normal	MRI-discrete lesions in the vicinity of dorsal parts of lateral ventricle corpora, paraventricular foci of demyelination?
18			0.24	Normal	CT-isolated tiny hypodense foci in midbrain and pons
19	N/A	7.9	N/A	Normal	MRI-choroid plexus cysts

[1]In Pt 13, the anti-streptolysin titer was 530 U ($n \leqslant 200$ U). WBC: White blood cells; CRP: C-reactive protein ($n \leqslant 5$ mg/mL); HV: Hyperventilation; N/A: Data not available; CT: Computed tomography; MRI: Magnetic resonance imaging; ESR: Erythrocyte sedimentation rate; EEG: Electroencephalography.

tant causes of headaches.

In the United States, migraine is a common neurological disorder, with about 18% of women and 6% of men suffering from the disease[19]. In 1988, it was postulated that the neurogenic inflammation due to acquired TG infection might be the cause of different types of headaches[14-16]. Several studies showed that the parasite harbors molecules that induce synthesis of proinflammatory cytokines because investigations performed *in vitro* showed proliferation of TG tachyzoites in HeLa cells and increased secretion and expression of monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α) and MIP-1β, cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) *via* mitogen-activated protein kinases[20-24]. The stimulation of human polymorphonuclear leukocytes by the TG antigen has been shown to upregulate MIP-1α and MIP-1β[22]. Cysteine-cysteine chemokines, MCP-1 and MIP-1α, may contribute to the recruitment of monocytes and lymphocytes[22], COX-2 catalyzes production of PGEs from arachidonic acid at inflammation sites[20], and PGE_2 may promote T_H type 2 immune responses by impairing the ability of locally maturing dendritic cells to produce proinflammatory cytokine interleukin-12 (IL-12)[25,26]. PGE_2 is also a potent suppressor of both monocyte antigen presenting function and T-cell expression of IL-2[27].

Studies by Kaciński *et al*[28] and Gergont *et al*[29,30] performed in a large cohort of children with migraine and episodic tension headaches showed a significant increase of serum IL-1β, IL-6, tumor necrosis factors (TNF)

and soluble TNF type I receptor levels compared with controls. It should be emphasized that investigations performed in women with acute TG infection (peripheral lymphadenopathy, IgM index > 0.7, specific anti-TG IgG titer exceeding 300 IU/mL, low avidity) showed a highly significant increase in serum IL-5, IL-6 and IL-10, while TNF-α level was not changed, which indicated a proinflammatory process and simultaneous anti-inflammatory reactions of the organism (increased level of IL-4, IL-10, IL-13) trying to counterbalance the excess of systemic pro-inflammatory activity[31]. The anti-inflammatory cytokines IL-4, IL-10 and IL-13 have also been detected during the interictal period in plasma of children with migraine and tension-type headache[32]. These data are consistent with the findings of Koseoglu *et al*[18] who in the group of 104 patients with migraine showed significantly increased serum anti-TG antibodies levels in 46 of them using the ELISA test compared to 26 seropositive results in 50 healthy individuals and 12 positive values in the group of 50 patients with headaches due to sinusitis. Moreover, finding of markedly increased concentrations of proinflammatory cytokines in the cerebrospinal fluid [MCP-1, IL-1ra, transforming growth factor beta 1 (TGF-β1)] and plasma (TNF, IL-1β, sTNF type I receptor, IL-6, sICAM-1) of children and adolescents with migraine and other types of headaches[33-35] corroborates further the earlier suggestion of Prandota[16] that those disturbances have been, at least in part, caused by a neurogenic inflammatory process that enhances production of cerebrospinal fluid. This explanation is also in line with the feeling of no discomfort reported by our eight IgG seropositive children with headaches, probably because intracranial pressure had not yet reached a clinically significant limit.

The presented reasoning is in line with the reports highlighting a possible role of inflammation in migraine pathophysiology[34,36-38]. Recent studies in migraineurs showed an increased production of matrix metalloproteinase-9 (MMP-9)[33,37] and TGF-β1[34], a multifactorial proinflammatory cytokine, and it was reported that NO in a concentration-dependent manner regulated MMP-9 activity secreted from macrophages[38]. It must be emphasized that NO is a key molecule in migraine and other vascular headaches responsible for cerebral and extracerebral cranial blood flow and arterial diameters and nociceptive processing[39-41]. Moreover, triptans (including sumatriptan, the golden standard in the treatment of acute migraine) showed some anti-inflammatory and immunomodulatory potentials[42-44]. For example, these drugs were found to directly inhibit pMMP-9 secretion by neutrophils[44], reduce the influx of leukocytes into the site of inflammation, formation of brain edema, and inhibit neuropeptides calcitonin gene-related peptide and substance P release[42]. All these processes may affect the development of immune processes responsible for reactivation of cerebral toxoplasmosis. Although sumatriptan indirectly inhibited peripheral blood mononuclear cells' natural killer (NK) cell activity[44] important for production of interferon gamma (IFN-γ)[45], it is known that only NK1.1(+) T-like cells, but not NK cells, negatively regulate the protective immunity against TG infection, possibly by producing IL-4 and suppressing heat shock protein 65 expression in the host macrophages[46-48]. According to these premises, it seems that tests detecting TG infection should be introduced on a routine base to the diagnostic process of children, adolescents and adults with headaches. Moreover, taking into consideration our previous and present clinical experience[14-16,49,50], we suggest treating these patients for 5-7 d with co-trimoxazole, the drug successfully used in patients with acquired immunodeficiency syndrome and cerebral toxoplasmosis[51,52]. Doses of the drug should be at the lowest recommended range because of the possible development of the Jarisch-Herxheimer reaction due to massive killing of the parasite associated with influx of foreign proteins into the host systemic circulation[57,58]. This may manifest as a transient and markedly increased intensity of headache and sometimes as aseptic meningitis[50,59-62].

In our patients, epilepsy, bronchial asthma, allergic rhinitis, recurrent upper airways infections, eustachitis and paranasal or ethmoid sinusitis were frequent comorbidities. These clinical disturbances are similar to the findings of Lateef *et al*[63] who reported asthma, hay fever and frequent ear infections as more common in children with headache, with at least 1 of these occurring in 41.6% of children with headache *vs* 25.0% of children free of headache. Aamodt *et al*[64] even suggested that both migraine and nonmigrainous headache are related to asthma, hay fever and chronic bronchitis. These suggestions may be partly supported by the *in vitro* finding that betamethasone, a glucocorticoid anti-inflammatory and immunosuppressant sometimes used in these clinical entities, increases invasion of TG tachyzoites to the host cells *in vitro*[65] and atopic dermatitis was frequently diagnosed in the seropositive ant-TG IgG children with headaches[16]. Note that recently it was reported that the parasite actively inhibits neuronal function in chronically infected mice[66] and histamine, known to be produced in increased quantities in atopic dermatitis and asthma, exerts anti-inflammatory effects and modulates microglia function[67,68]. Histamine enhanced the secretion of T_H2 cytokines, such as IL-4, IL-5, IL-10 and IL-13, and inhibited the production of T_H1 cytokines IL-2 and IFN-γ, as well as modulated the cytokine network through up-regulation of prostaglandin E_2 and nitric oxide (NO)[68]. It must be added that two of our seropositive children had epilepsy and it was reported that chronic TG infection could be a cause of cryptogenic epilepsy[69-71] and several antiepileptic drugs have anti-toxoplasma activity (Table 5). Moreover, strabismus observed in Pt 17 also may be due to congenital toxoplasmosis[72].

The value of computed tomography in diagnosis of headaches was very limited in our patients, while MRI studies appeared to be useful in showing brain abnormalities in 7 out of 11 studied individuals. These findings are in agreement with the results of other authors[73,74]

Table 5 Drugs tested for *in vitro* activity against Toxoplasma gondii (according to Jones-Brando *et al*[52]; with own modification)

Drug	Solvent	ID50[1] (μg/mL)	TD50[2] (μg/mL)	TI[3]
Valproic acid	Ethanol	4.5	62.4	13.9
Sodium valproate	Ethanol	4.1	52	12.7
Carbamazepine	Ethanol	72	100	1.3
Lithium carbonate	1 N HCl	> 100	> 100	
Haloperidol	Ethanol	5.6	103	18.4
9-OH-Risperidone	Tartaric acid	20.1	134	6.7
Risperidone	Tartaric acid	74	129	1.7
Fluphenazine HCl	Toxo CGM	3.5	17.9	5.1
Clozapine	Ethanol	5.8	20	3.4
Olanzapine	DMSO	33.2	100	3
Chlorpromazine HCl	DMSO	2.6	6	2.3
Quetiapine fumarate	DMSO	18.6	33	1.8
Trimethoprim	DMSO	5.3	63.8	12.1

[1]Median inhibitory dose, a measure of tachyzoite inhibition. [2]Median toxicity dose, a measure of cytotoxicity. [3]Therapeutic index, a measure of efficacy determined by TD_{50}/ID_{50} ratio. Valproic acid at a concentration of 1 μg/mL inhibited 7% of the tachyzoites and trimethoprim at 3.2 μg/mL produced 2% inhibition, but the combination of these two compounds at those concentrations resulted in a potentiating effect inhibiting 55% of the tachyzoites. Recently, Fond *et al*[53] also reported that other antipsychotic drugs, such as amisulpride, cyamemazine, levopromazine, loxapine, tiapride and zuclopenthixol *in vitro* exerted anti-toxoplasmatic activity in the range of 0.19 to 1 μmol/L concentrations. The inhibitory concentration 50 (IC_{50}) for the last preparation was 8 ± 1.8 μmol/L while its serum levels varied from 0.01-0.12 μmol/L[54], but antipsychotic drugs usually achieve much higher and persistent concentrations in the brain tissue[55]. In human fibroblast cell cultures, IC_{50} for fluphenazine, thioridazine and trifluoperazine against developing tachyzoites of the parasite RH strain was 1.7, 1.2 and 3.8 μmol/L, respectively[56]. DMSO: Dimethylsulfoxide; Toxo CGM: Toxoplasma cell growth medium; TI: Therapeutic index; TD: Median toxicity dose; ID: Median inhibitory dose.

who suggested that neuroimaging procedures should not be routinely ordered in the initial diagnosis of types of headaches, recent onset of severe headaches, neurological dysfunction and on demand by parents or physicians[74].

Twelve of 178 children with recurrent headaches had slightly increased but not significant serum anti-Toxo IgM concentrations ranging from 0.1 to 0.3 IU/mL. This may result in part from different proteins from various TG strains and subclinical amounts and of the parasite antigens responsible for the generation of the host antibodies[75]. It should be noted, however, that at present diagnostic tests used for estimation of TG IgG, IgM and IgA seropositivity are not fully sensitive and specific and various methods of serum sample preservation and elaboration[76], as well as a diseases state of the host (*e.g.*, oxidative stress and resulting protein oxidation), may also affect both these parameters[77,78]. One may therefore suggest that such patients should receive a 5-7 d pilot treatment to avoid costly diagnosis and hospitalization because it was found that in animals antiparasitic treatment suppressed production and avidity of TG-specific antibodies[79].

In summary, 19 (10.67%) out of 178 studied young children and adolescents had specific serum anti-TG IgG antibodies and high index of avidity suggesting chronic infection. In our patients, peripheral lymphadenopathy, which would be of help in the diagnosis of acquired toxoplasmosis, was only observed in one child. On admission, the main clinical symptoms were headaches affecting the frontal, parietal and occipital areas. Almost all of the children had pets that may have been the source of infestation. In order to maintain a successful school performance as well as relationship with family and peers, primary care physicians should be aware of the probability of cerebral toxoplasmosis and therefore tests for TG infection must be taken into consideration during a routine diagnostic process of patients suffering from recurrent headaches because it is an underestimated condition, even in neurology practice[80].

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COMMENTS

Background

Headaches in both children and adults are very frequent and diagnosis often requires hospitalization associated with very expensive diagnostics. At present, in immunocompetent individuals Toxoplasma gondii (TG) infection is believed to be asymptomatic and in pediatrics/neurology books there is no suggestion of performing tests to search for infection with this parasite.

Innovations and breakthroughs

This work estimated the seroprevalence and characteristics of TG infection in 178 children admitted consecutively to the Pediatric Neurology Department because of recurrent headaches.

Applications

This work will help pediatricians and family physicians to check such patients for TG infestation.

Peer review

Prandota *et al* report an analysis of 178 patients admitted to the neurology service. Out of this cohort, 19 patients tested positive for TG infection. This is a well written article. It has high priority for publication in your esteemed journal.

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Rituximab for troublesome cases of childhood nephrotic syndrome

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Abstract

Nephrotic syndrome (NS) is the most common glomerular disease of childhood. Steroid-dependent and steroid-resistant nephrotic syndrome present challenges in their pharmaceutical management; patients may need several immunosuppressive medication for optimum control, each of which medication has its own safety profile. Rituximab (RTX) is a monoclonal antibody that targets B cells and has been used successfully for management of lymphoma and rheumatoid arthritis. Recent clinical studies showed that rituximab may be an efficacious and safe alternative for the treatment of complicated nephrotic syndrome. In this review article, we aim to review the efficacy and safety of RTX therapy in nephrotic syndrome. We reviewed the literature pertaining to this topic by searching for relevant studies on PubMed and Medline using specific keywords. The initial search yielded 452 articles. These articles were then examined to ensure their relevance to the topic of research. We focused on multicenter randomized controlled trials with relatively large numbers of patients. A total of 29 articles were finally identified and will be summarized in this review. The majority of clinical studies of RTX in complicated pediatric NS showed that rituximab is effective in approximately 80% of patients with steroid-dependent NS, as it decreases the number of relapses and steroid dosage. However, RTX is less effective at achieving remission in steroid-resistant NS. RTX use was generally safe, and most side effects were transient and infusion-related. More randomized, double-blinded clinical studies are needed to assess the role of RTX in children with nephrotic syndrome.

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Key words: Rituximab; Pediatric; Nephrotic syndrome; Efficacy; Safety

Core tip: Nephrotic syndrome (NS) is the most common pediatric glomerular disease. Although outcomes are favorable, the treatment of complicated nephrotic syndrome can be challenging. Rituximab (RTX) offers a safe and effective alternative to current immunosuppressive therapies for complicated cases of NS. The best outcomes are seen in patients with steroid-dependent NS who have failed to respond to multiple therapies. However, the benefits of RTX therapy are limited in patients with steroid-resistant nephrotic syndrome. Successful RTX therapy induces prolonged remission and enables discontinuation of other medications without increasing the risk of infection and other adverse events.

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INTRODUCTION

Nephrotic syndrome (NS) is the most common glomerular disease of childhood. Although long-term outcomes are favorable, a significant proportion of patients de-

velops steroid-dependent disease or frequent relapses and is at high risk for prolonged steroid exposure. Such patients develop steroid toxicity, including hypertension and growth arrest[1]. Approximately 10%-20% of patients with nephrotic syndrome do not respond to steroid therapy (steroid-resistant disease); such patients are at a significant risk of developing end-stage renal failure[2]. Currently used second line agents for the long-term treatment of patients with complicated nephrotic syndrome, such as calcineurin inhibitors (CNI), mycophenolate mofetil (MMF), levamisole and others, suppress or control proteinuria. However, these immunosuppressive agents have their own adverse systemic effects, including nephrotoxicity with CNI; Gastrointestinal upset with MMF, and leukopenia with levamisole. No high-quality randomized controlled trials have performed head-to-head comparisons to establish the superior efficacy or safety of one immunosuppressive agent over its counterparts. None of these agents "cure" the disorder, and their exact mechanism of action in nephrotic syndrome is still largely unknown. Most patients experience a recurrence of proteinuria or nephrotic syndrome after medications are discontinued.

Rituximab (RTX) is a chimeric, monoclonal anti-CD20 antibody that targets B lymphocytes and causes Fc-mediated cell lysis[3,4]. It has been increasingly used in recent years to treat various immunological and non-immunological diseases. Over the last 7 years, a number of groups have shown that the depletion of CD20-positive B lymphocytes with the use of the chimeric monoclonal antibody RTX can lead to long-lasting remission of nephrotic syndrome due to minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS)[5-8].

Many years ago, Shalhoub *et al*[9] proposed that T-cells are the main culprit in the pathogenesis of nephrotic syndrome. However, past evidence has shown that CD23 (a B-cell marker), is elevated during clinical relapse in steroid-dependent nephrotic syndrome[10]. It is well known that B-cells play multiple physiological roles in immune system function. These cells are the precursors of antibody-producing cells (*i.e.*, plasma cells). They also serve as antigen-presenting cells and are vital to cytokine production and secretion of the co-stimulatory signals that are required for CD4 activation.

Treatment with RTX does not appear to cure nephrotic syndrome. Patients often relapse several months following treatment with RTX (Table 1).

This review article provides insight regarding the efficacy of RTX in steroid-dependent nephrotic syndrome and examines adverse events related to the use of RTX in patients with nephrotic syndrome.

For a better understanding of this review, it is prudent to clarify the definitions of a few key terms prior to delving into more detail regarding the core topic. Steroid dependent-nephrotic syndrome (SDNS) has been defined according to the Arbeitsgemeinschaft für Pädiatrische Nephrologie standard definition as at least two relapses with alternate-day prednisone treatment (40 mg/m^2) or within 14 d after stopping this treatment. Frequent relapsing nephrotic syndrome has been defined as 2 or more relapses within 6 mo and/or 4 or more relapses within 12 mo. Steroid-resistant nephrotic syndrome has been defined as a lack of remission (urine albumin nil/trace by dipstick for 3 consecutive days) despite therapy with prednisone at 2 mg/kg per day for 4 wk.

RESEARCH

We searched medical databases including PubMed and Medline to identify studies that addressed the efficacy and safety of RTX in children. The search words were "Nephrotic syndrome", "Steroid dependent", "Steroid resistant", "Children", "Rituximab and adverse effect" and "RTX". We included randomized clinical trials and case series. The full texts of all potentially relevant abstracts were retrieved for further review.

STEROID DEPENDENT NEPHROTIC SYNDROME

The literature review showed that past evidence favors the use of RTX as a promising agent for maintaining remission in SDNS. RTX seems to be effective in terms of reducing relapses and reducing the required steroid dose.

In a recent multicenter, double-blind randomized controlled trial, Iijima *et al*[11] showed that RTX is effective and safe in children with SDNS and frequent relapsing nephrotic syndrome. A total of 48 patients were enrolled (24 received RTX, and 24 received placebo). Patients receiving RTX had a longer median relapse-free period compared to placebo (267 d *vs* 101 d; P value < 0.0008). Ten patients (42%) treated with RTX developed at least one serious adverse event, compared to 6 patients in the placebo group (25%); this difference was not statistically significant, with a P value of > 0.36 (Table 1).

Guigonis *et al*[5] demonstrated the efficacy of RTX in maintaining prolonged and effective remission in 22 children with steroid- or cyclosporine-dependent nephrotic syndrome. One or more immunosuppressive treatments could be withdrawn in 19 of these 22 patients (85%), with no relapse of proteinuria and without increasing the doses of other immunosuppressive drugs (Table 1).

Gulati *et al*[7] reported the efficacy and safety of RTX in SDNS refractory to standard therapy in a 3-year long prospective cohort study that was conducted in tertiary care centers in India and the United States. After steroid-induced remission, RTX was given at a mean dose of 400 ± 20.7 mg/wk for 2 wk. After 12 mo, remission was sustained in 20 (83.3%) patients. The mean number of relapses before therapy decreased significantly (P = 0.001). At a mean follow-up of 16.8 ± 5.9 mo (range, 12 to 38 mo), remission was sustained in 17 (71%) patients (Table 1).

Kemper *et al*[8], in a study of RTX in 37 children with steroid-dependent disease, showed that 26 patients (70.6%) remained in remission after 12 mo of treatment. In 29 children with prolonged follow up (for > 2 years), 12 children maintained remission (41%). By giving addi-

Table 1 Summary of published studies of rituximab in childhood nephrotic syndrome

Ref.	Demographics	Histologic findings	No. of doses	Outcome
Gulati *et al*[7]	SDNS Age (mean): 12.7 ± 9.1 yr 33 SRNS M: 17 F: 16 Age (mean): 11.7 ± 2.9 yr M: 19 F: 5	SRNS 17 MCD (51%) 16 FSGS (49%) SDNS 12 MCD (50%) 2 FSGS (8%) 2 Mesangial hypercelluarity (8%) 8 No biopsy (33%)	SDNS 2 doses SRNS 4 doses	SDNS: 1 yr 20 remission; after 1 yr, 17 still in remission SRNS at 9 mo: 9 CP, 7 PR, 17 NON After 1 yr: 7 CR, 8 PR
Kemper *et al*[8]	37 SDNS M: 25 F: 12 Age (mean): 4.4 ± 3.1 yr		1-4 doses	After 1 yr, 26 patients (70.3%) remained in remission After 2 yr, 12 patients (41%) remained in remission
Guignonis *et al*[5]	22 SDNS Age: 6.3-22 yr			Immunosuppressive withdrawn in 19 patients with no relapse of proteinuria (85%)
Iijima *et al*[11]	48 SRNS RTX 24 (intervention) Placebo 24 (control)		4 infusions weekly for 4 doses	
Tellier *et al*[12]	18 SDNS M: 9 F: 9 Age (median): 2.8 yr (1.6-7.4)		One to four infusions	22% had remission without relapse and increased duration of remission
Kimata *et al*[17]	5 SDNS Age (median): 6.3 yr (0.9-8.4)		Four doses every 3 mo	Number of relapses = 0 after the last dose of RTX
Ravani *et al*[13]	46 PT SDNS Age (mean): 9.9 ± 4.3 yr M: 29 F:17		One to five	Probability of remission after rituximab At 6 mo: 48% At 1 yr: 20% At 2 yr: 10%
Magnasco *et al*[20]	SRNS 31 Median: 7.9 ± 4.1 yr M: 19 F: 12 Control group: 15 Intervention: 16	19 FSGS (61%) 7 MCD (23%) 5 no biopsy (16%)		No change in proteinuria
Prytula *et al*[16]	SDNS/FR 28 Age (median): 4 yr (18 mo -17 yr) M: 25 F: 3 SRNS 27 Age (median): 3 yr (18 mo-11 yr) M: 17 F: 10	SDNS MCD 17 FSGS 5 Mesangial proliferation 2 IgM nephropathy 1 No biopsy 3 SRNS 11 MCD 11 FSGS Mesangial proliferation 3 IgM nephropathy 1 No Biopsy 1		SDNS CR: 17 (61%) PR: 8 (28% NR: 3 (11%) SRNS CR 6 (22%) PR 12 (44%) NR 9 (34%)

CR: Complete remission; PR: Partial remission; NR: Nephrotic syndrome; NONE: No response; M: Male; F: Female; SDNS: Steroid-dependent nephrotic syndrome; SRNS: Steroid-resistant nephrotic syndrome; MCD: Minimal change disease; FSGS: Focal segmental glomerulosclerosis.

tional 2-4 courses to 19 patients, the number of patients who maintained long-term remissions increased to 20 (69%) (Table 1).

In a retrospective analysis, Tellier *et al*[12] assessed the long term outcome of RTX therapy for SDNS in 18 patients with a mean follow up of 3.2 yr. The children received one to four infusions of RTX during the first course of treatment, and subsequent infusions were given upon CD19 cell recovery (CD19 > 1%). A total of 22% of patients maintained remission without relapse. Ravani *et al*[13] performed a study to assess whether RTX can be safely and repeatedly used as prednisone and CNI-sparing therapy in children with dependent forms of idiopathic nephrotic syndrome. Approximately 46 patients were enrolled in the study; all received one to five RTX infusions. The probability of remission without the use of immunosuppressants was 48% and 20% at 6 and 12 mo, respectively, after RTX treatment (Table 1).

Sun *et al*[14] also showed that RTX was effective in maintaining remission in children with nephrotic syn-

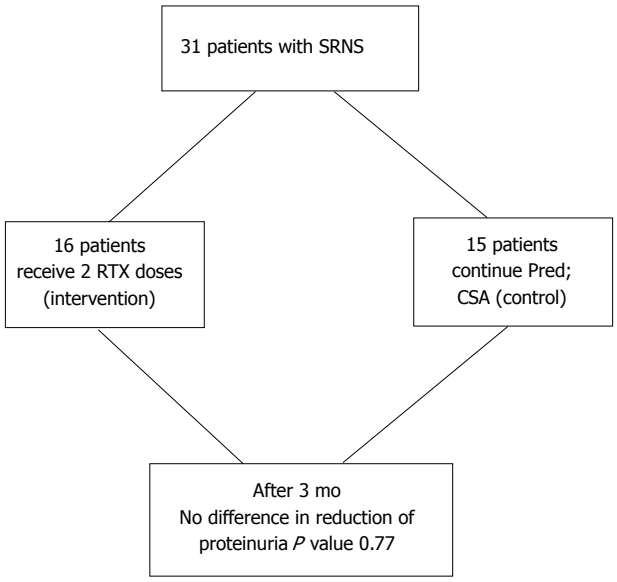

Figure 1 Rituximab *vs* conventional treatment in steroid resistant nephritic syndrome. SRNS: Steroid resistant nephritic syndrome; Pred: Prednisone; CSA: Cyclosporine A; RTX: Rituximab. From ref.[20].

drome. RTX treatment led to a statistically significant reduction in steroid dosage ($P = 0.014$); the number of recurrences was also significantly reduced ($P < 0.001$) with RTX therapy (Table 1).

A Scottish study involving seven children[15] with steroid-dependent disease showed that two doses of RTX (750 mg/m^2) given 2 wk apart were effective in maintaining remission in six children (Table 1).

Prytula *et al*[16], in a multicenter questionnaire study, demonstrated that RTX therapy could elicit a response rate of 82 % in SDNS.

It is important to note that different studies have proposed differing numbers of initial RTX infusions. Hence, based on past evidence, it is difficult to comment on the optimum number of RTX infusions. Kemper *et al*[8] noted that 3-4 initial infusions of RTX were not associated with better or prolonged remission rates, compared to 1-2 initial RTX infusions.

The optimal RTX treatment strategy is also unclear. In a few past studies, RTX was given prophylactically upon B-cell recovery; in other studies, RTX therapy was begun only in cases of relapse. Tellier *et al*[12] showed that the time between two relapses was significantly shorter in patients who were retreated upon B-cell recovery compared to those who were not re-treated. This indicates that pre-emptive treatment at the time of B-cell recovery may help to maintain prolonged remission.

The use of RTX at regular intervals is another therapeutic approach. Kimata *et al*[17] demonstrated that RTX infusion at 3-mo intervals for a total of four doses is an effective strategy in maintaining remission and steroid-free periods. In this study, the annual number of relapses before administration was 1.4 (1.1-3.5) times/year. After administration, the number of relapses had decreased to 0.0 (0.0-0.0) times/year. Median steroid dosage before administration was 0.80 (0.23-0.96) mg/kg per day but was 0.00 (0.00-0.00) mg/kg per day after administration. All changes were statistically significant at $P < 0.05$.

Fujinaga *et al*[18] gave a single infusion of RTX (375 mg/m^2) to 29 children with steroid/calcineurin inhibitor-dependent nephrotic syndrome. After RTX, 16 patients received MMF, and 13 patients received cyclosporine (CsA) as maintenance therapy. Cyclosporine was superior to MMF in maintaining remission. Treatment failure occurred more frequently in the MMF group (7/16) than in the CsA group (2/13). The rate of sustained remission was also significantly higher in the CsA group than in the MMF group ($P < 0.05$).

The evidence described above shows that RTX is an effective agent in children with SDNS/frequently relapsing disease, both in terms of maintaining prolonged remission and decreasing steroid exposure. However, common drawbacks of most such studies have been the small sample size and the lack of patients with nephrotic syndrome who were receiving conventional treatments or placebo (*i.e.*, control groups). This highlights the need for more robust randomized controlled studies to assess the efficacy, safety and optimal dosing patterns of RTX.

STEROID-RESISTANT NEPHROTIC SYNDROME

The treatment of steroid resistant nephrotic syndrome is a challenge for clinicians. The disease has genetic causes in one-quarter to one-third of such patients[19]; patients with genetic aberrations do not benefit from immunosuppressive treatment.

In a randomized controlled study conducted between 2007-2010, Magnasco *et al*[20] evaluated RTX in 31 children with SRNS and a median age of 8 years (2-16 years). All patients were negative for the nephrotic syndrome 2 (NPHS2) and WT-1 mutations. Renal biopsy revealed that focal segmental glomerulosclerosis was the most common histological diagnosis in 19 patients (61%), followed by minimal change disease in 7 patients (23%). Patients were divided into 2 groups; the control group included 15 patients who continued conventional immunosuppressive treatment with prednisone and cyclosporine, and the interventional group included 16 patients who continued treatment with prednisone and cyclosporine, as well as receiving 2 doses of RTX. RTX did not reduce proteinuria at 3 mo, and the results of the study did not support the addition of RTX to prednisone and calcineurin inhibitors in children with SRNS (Figure 1). Histological diagnosis did not affect outcomes or response to RTX treatment (Table 1).

Gulati *et al*[7] assessed the efficacy of RTX in 33 pediatric patients with SRNS (24 with initial resistance and 9 with late resistance). Treatment consisted of 4 weekly doses of RTX (375 mg/m^2 each). All patients but five were screened for NPHS1 and NPHS2. Nine patients (27.2%) achieved complete remission, 7 patients (21.2%) achieved partial remission, and 17 patients (51.5%) had no response. The median time to response was 32 days

(range, 8 to 60 d) after the last dose of RTX. At the 6-mo follow up, all 16 patients remained in remission (complete or partial). The remission rate was higher in patients with minimal change disease (64.7%) than in those with FSGS (31.2%; $P = 0.08$) (Table 1).

Prytula *et al*[16] conducted a multicenter, questionnaire-based study to assess the initial response to RTX in children with SRNS and determined the initial response to be 44 %. However, a randomized study conducted by Magnasco *et al*[20] showed no benefit from RTX therapy.

In an assessment of five children with SRNS, Bagga *et al*[21] showed that 4 RTX doses of 375 mg/m^2 were effective in achieving complete remission in four patients, while one patient achieved partial response. The mean ratio of urinary protein to creatinine was 8.3 at baseline and 0.8 at follow-up ($P = 0.02$, by analysis of variance), and the mean serum albumin level rose from 1.4 to 3.4 g per deciliter ($P < 0.01$).

It can be inferred from the findings of the studies included here that RTX therapy is less efficacious in the treatment of SRNS than in the steroid-dependent/frequently relapsing form of the disease. Cravedi *et al*[22] attributed the low serum level of RTX in patients with SRNS to urinary loss of RTX in these patients during the nephrotic state of the disease (not during remission). In addition, mutations in genes that encode an essential protein in the slit diaphragm of the glomerular filter play an important etiological role in patients with SRNS.

SAFETY

RTX has been used over the last few years for the treatment of complicated cases of pediatric nephrotic syndrome and has shown promising clinical efficacy.

However, questions regarding its safety and long-term effects in this particular group of patients have remained unanswered. A few previous uncontrolled studies have shown that RTX was well tolerated in pediatric patients with the steroid-dependent and resistant forms of nephrotic syndrome. Most adverse events were mild infusion-related reactions and resolved either spontaneously or with intramuscular epinephrine. Very few patients developed severe anaphylactic reactions, hypotension or cardiac arrhythmia[5]. The pathogenesis of such reactions is still unclear but may be related to cytokine release from the destroyed B cells.

Gulati *et al*[7] treated 33 pediatric patients with RTX (24 with SDNS, 9 with SRNS); only 4 patients (12%) developed infusion reactions (3 had chills and 1 had myalgia).

Another study of 30 patients from France[6] showed that 14 patients (47%) developed infusion-related reactions that were mild and did not require RTX discontinuation. Kemper *et al*[8] treated 37 patients with RTX, and only 2 patients developed itching at the first infusion. It is difficult to comment on the reasons for the differences in the number of adverse reactions noted among different studies. Bitzan *et al*[23] reported a patient with an RTX-related lung injury that resolved without any serious sequelae. There are more than 30 published reports (with 62 cases) of delayed pulmonary toxicities after RTX treatment. These have mostly been reported in adult patients with hematologic malignancies. RTX-related pulmonary toxicities can be serious and carry a significant mortality rate. Delayed pulmonary toxicities have not been reported in other clinical studies of pediatric nephrotic syndrome patients treated with RTX. However, Kamei *et al*[24] reported mild acute respiratory events during RTX infusion in 10/28 patients (36%). Sellier-Leclerc *et al*[6] reported adverse reactions in two nephrotic syndrome patients receiving RTX. Of these, one developed transient severe neutropenia, and the other developed transient thrombocytopenia. In general, hematological adverse events were uncommon and had no serious clinical effects. Serious infections were rare. Guigonis *et al*[5] reported that in a multicenter study of RTX in 22 patients, one patient developed Pneumocystis carinii pneumonia (PCP) despite PCP prophylaxis. This patient was also receiving tacrolimus and corticosteroids at the time of PCP infection.

In a multicenter questionnaire-based study, Prytula *et al*[16] showed that 3/70 patients receiving RTX developed serious infections; one patient had agranulocytosis with sepsis, and two patients had pneumonia.

Kari *et al*[25] studied 4 patients receiving RTX for SRNS and reported that one patient developed clinical peritonitis. Of interest, a few patients developed fever of unknown origin after RTX without a clear microbial cause[5].

Data regarding the safety of RTX are not limited to pediatric patients with nephrotic syndrome. Several other research reports have also delved into the safety aspects of RTX therapy in adults. For example, RTX was well tolerated and generally found safe among patients with non-Hodgkin's lymphoma, and very few patients experienced serious adverse events. Data from the German national registry[26] revealed that out of 370 adult patients who received RTX for various immunological diseases, the overall rate of serious infections was 5.3 per 100 patient-years during RTX therapy. Opportunistic infections were infrequent across the whole study population and mostly occurred in patients with systemic lupus erythematosus. There were 11 deaths (3.0% of patients) after RTX treatment. Sellier-Leclerc *et al*[27] reported a case of fulminant myocarditis after 4 mo of RTX infusion in a child with nephrotic syndrome who survived after receiving a heart transplant.

Progressive multifocal leukoencephalopathy is a serious demyelinating disease of the central nervous system arising from reactivation of the John Cunningham virus. It has been reported in various immunologic diseases[28] and with the use of various biological agents, including RTX. This is has not been reported in pediatric patients with nephrotic syndrome who were treated with RTX.

RTX has shown a reasonable safety profile in other pediatric autoimmune diseases. A systemic analysis of the use of RTX in children with idiopathic thrombocytope-

nic purpura[29] showed that in 11 studies (190 patients), a total of 78 (41.1%) patients experienced adverse events.

CONCLUSION

This review allows us to conclude that RTX appears to be an effective therapy that maintains remission in children with SDNS who failed to respond to other medications. The role of rituximab in children with SRNS needs to be evaluated further, as there are conflicting results regarding its efficacy in this group of patients. More randomized controlled studies are before recommending rituximab as a rescue therapy in children with SRNS. RTX seems to be safe in children with nephrotic syndrome, and the main adverse events are related to infusion reactions. However, close monitoring for long-term side effects is recommended.

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