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Study protocol for a pilot randomised controlled trial evaluating the effectiveness of oral trehalose on inflammatory factors, oxidative stress, nutritional and clinical status in traumatic head injury patients receiving enteral nutrition

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ABSTRACT

Introduction In traumatic brain injury (TBI) patients, inflammatory processes and oxidative stress have been linked to the development of neurodegenerative diseases, disability, increased rate of muscle catabolism, malnutrition, hospital stay and mortality. Previous in vitro and in vivo studies have shown that trehalose can decrease inflammatory and oxidative factors. Therefore, the present study was designed to evaluate the effect of oral trehalose consumption on this marker in critically ill TBI patients at intensive care unit (ICU).

Methods and analysis This study is a pilot randomised, prospective and double-blind clinical trial. The study sample size is of 20 (10 patients in each group) TBI patients aged 18–65 years at ICU. Randomisation is performed by permuted block randomisation method. The allocation ratio is 1:1. An intervention group will receive 30 g of trehalose instead, as a part of the carbohydrate of daily bolus enteral feeding and the control group will receive standard isocaloric hospital bolus enteral feeding for 12 days. The inflammatory factors (C reactive protein, interleukin 6) and oxidative stress markers (glutathione, malondialdehyde, superoxide dismutase, pro-oxidant–antioxidant balance, total antioxidant capacity) will be measured at the baseline, at the 6th day, and at the end of the study (12th day). Sequential Organ Failure Assessment, Acute Physiology and Chronic Health Evaluation II, Nutrition Risk in the Critically ill scores, 28-day mortality, anthropometric assessments and the clinical and nutritional status will be measured. Each patient's nutritional needs will be calculated individually. The statistical analysis would be based on the intention to treat.

Ethics and dissemination The vice-chancellor of the research centre of Mashhad University of Medical Sciences is sponsoring this study. IR.MUMS.MEDICAL.REC.1400.113.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first clinical trial designed to determine the effect of trehalose consumption in traumatic brain injury patients at intensive care unit.
⇒ This pilot randomised controlled trial protocol is developed according to the Standard Protocol Items: Recommendations for Interventional Trials guidelines.
⇒ Blood transfusion may affect the biochemistry outcomes.

Trial registration number Iranian Registry of Clinical Trials (IRCT) Id: IRCT20210508051223N1, Registration date: 26 July 2021.

INTRODUCTION

Trauma to the head can lead to a wide range of mild to severe injuries, disabilities and death.[1] The treatment and rehabilitation processes of the traumatic brain injury (TBI) patient impose a substantial burden on the healthcare system and indirectly on society and the economy.[2 3] There is an incidence peak of TBI in young adults.[4] Damage to the head leads to the primary (extrinsic impact and direct damage to the brain tissue) and secondary injuries (molecular, mitochondrial, chemical and inflammatory disorder such as acute phase response and systemic inflammatory response syndrome).[5 6] The breakdown of the blood–brain barrier, neuroinflammation and ischaemic injury can cause many changes in brain metabolism.[7–10] Secondary injuries of TBI lead to the

following disorders: disruption of cerebral blood flow, disruption of metabolism and excessive secretion of excitatory neurotransmitters and enzymes. These phenomena are linked to hypoxia, increased energy expenditure and increased production of reactive oxygen species.[5 11] Therefore, overproduction of inflammatory mediators, free radicals and the activation of cell death signalling pathways associated with brain cell self-digestion. These conditions can lead to poor treatment outcomes.[5] As a result of TBIs, numerous patients are living with disabilities.[12] In addition to medication, the use of supplements or nutrients that help control inflammatory and oxidative conditions can be useful.

In recent years, attention to trehalose has been increasing. Trehalose is a natural non-reducing disaccharide (O-α,-D-glucopyranosyl-[1→1]-α-D-glucopyranoside) that is found in nature (plants, insects, yeast, fungi, invertebrates and bacteria).[13–15] In human diet, some food such as mushrooms, honey, shell fish, beer wine and bread contain trehalose.[16] Trehalose will not easily interact with proteins, acid, α-glucosidase and other biological molecules.[17] Because of the existing glucoside α, α–1, 1 glucoside bond between two glucose units, trehalose has high chemical stability and maintains a glassy (amorphous) structure under a wide range of environmental conditions such as potential of hydrogen (pH) (3.5–10), low temperature, dehydration and other extreme environmental stresses.[18 19] The energy from a glycosidic bond is only 1 kcal/mol.[20]

Previous studies have demonstrated that trehalose might be involved in cellular autophagy.[14 21] Autophagy is one of the essential cell mechanisms that play the role of 'housekeeping'. The process of autophagy includes recycling and destroying accumulated proteins, cleansing of damaged organs, regulation of growth and ageing, cell differentiation, and defence versus pathogens and nutritional starvation.[22] In some human disorders, such as neurodegenerative and metabolic diseases and infections, autophagy has been irregular.[23]

The difference between trehalose and other saccharides is its protective action against environmental stressors. In vivo and in vitro studies have shown that trehalose can inhibit inflammatory responses to endotoxic shock.[11 16 24] According to an in vitro study performed on human eye cells, the following results were reported. Trehalose is effective in autophagy by increasing the levels of lysosomes while maintaining their PH. Reducing inflammation by reducing inflammatory cytokine levels such as interleukin (IL)-6, IL-8 and tumor necrosis factor-α (TNF-α) was also one of this study results.[25] In another study on rats with spinal cord injury, the use of trehalose led to a decreation in the level of inflammatory factors IL-1β and TNF-α as well as malondialdehyde (MDA) and nitric oxide.[26] Several animal studies have shown the beneficial effects of trehalose on some diseases such as diabetes,[21 25 27 28] Parkinson,[29] Alzheimer's,[30] atherosclerosis and on some conditions such as cerebral ischaemia,[31] hepatic and pulmonary oedema,[11] insulin resistance and metabolic syndrome.[32] Yoshizane *et al* in 2020 reported that trehalose is helpful in lower postprandial blood sugar in healthy humans.[33] Most human studies have been related only to preventing these conditions in healthy human. However, they have shown positive results.[20 34] The purpose of the present study is to determine the effect of trehalose on inflammatory cytokine and oxidative factors levels, in critically ill TBI patients under enteral feeding at intensive care unit (ICU).

RATIONALE

High level of inflammation and oxidative stress in TBI patients is associated with poor treatment outcomes, disability and high rate of mortality.[5 12] Furthermore, patients who admitted to the ICU are at risk for malnutrition. Therefore, good nutritional support is critical and beneficial for them.[35 36] Many studies have confirmed the beneficial anti-inflammatory and antioxidative effects of trehalose on various diseases but still no clinical trial studies have been performed on patients, particularly head trauma patients. The results of a study performed on male rats with endotoxic shock suggested that using trehalose is a secure biophysics approach to adjusting inflammatory response in critically ill patients.[11] We hypothesised that trehalose would decrease inflammation and oxidative stress, improve clinical and nutritional status. Accordingly, the present study was designed to determine the efficacy of administration trehalose in critically ill TBI patients under enteral nutrition therapy at ICU. This study is a phase II randomised controlled trial (RCT), and enrolment started in May 2022 and will be almost finished in August 2022. The trial procedures are expected to be completed by the end of September 2022.

METHODS AND MATERIALS

Trial design

This pilot study protocol describes the design of a parallel-group RCT (1:1 ratio) to investigate the effect of 30 g trehalose consumption in TBI patients. The framework of this study will be superiority. Allocation concealment will be considered using opaque-sealed sequentially numbered envelopes.

Trial population

The study participants will include 20 critically ill TBI patients at ICU. TBI will be diagnosed according to CT scan or MRI findings by a neurosurgeon. On the admission of patients in ICU, one person will be responsible for screening the eligibility of patients based on the inclusion, non-entry and exclusion criteria.

Inclusion criteria

1. Critically ill TBI patients admitted to ICU.
2. 18 years≤age≤65 years.
3. Stable haemodynamic and metabolic conditions in the first 24–48 hours.

4. Glasgow Coma Scale (GCS)≥7.
5. Having antral feeding.
6. Lack of intolerance to food sources containing trehalose such as mushroom.
7. Willingness to cooperate and signing the informed consent form after full knowledge of the objectives and method of the study by the patient or legal guardian.

Non-entry criteria

1. Head trauma patients who have been on nil per os for more than 48 hours (not allowed to receive food).
2. Patients who are transferred from other ICUs after 1 week.
3. Having a history of cancer, autoimmune diseases and congenital metabolic diseases.
4. Pregnancy and lactation.

Exclusion criteria

1. Request to stop the study by patients' legal guardian.
2. Being under enteral nutrition for less than 3 days.

Adherence to the intervention

Adherence to the intervention will be checked by daily nutritional visit, evaluation of clinical signs and enteral feeding tolerance. The compliance rate of trehalose consumption will be calculated according to the following formula and poor compliance will be considered less than 75%.[37 38] Compliance rate=(the powder weight used/the medication weight expected)×100.

where 'the medication weight used' is =(weight dispensed–weight returned). In this study, we want to present trehalose to participants through bolus enteral feeding. Therefore, those mild head trauma patients who are less than 3 days on enteral feeding will be excluded.

The intervention group

Participants will be randomised to the intervention group or control group. During 12 days, patients in the intervention group will receive 30 g of trehalose instead as a part of the carbohydrate of daily enteral feeding. Their feeds will be administered by the bolus method with 6–7 feeds. Infusion value will be 200–400 mL per each meal.[39] Nutritional information of enteral feeding components shows in table 1. The inflammatory factors and oxidative stress markers will be measured at beginning and end of study. Blood sugar, lipid profile, Sequential Organ Failure Assessment (SOFA) Score, Acute Physiology and Chronic Health Evaluation II (APACHE II), Nutrition Risk in the Critically ill (NUTRIC) Score, GCS, 28-day mortality, anthropometric assessments, clinical and nutritional status will be measured during the study. Each patient's macronutrients and micronutrients needs will be calculated individually based on The European Society for Clinical Nutrition and Metabolism (ESPEN) guideline.[40]

The control group

The control group will receive standard isocaloric hospital enteral feeding over 12 days. Exactly similar to the intervention group, inflammatory and oxidative stress factors, clinical and nutritional assessments at the same time will be measured.

Table 1 Nutritional information of enteral feeding

Compounds	Per 2000 mL
Energy (kcal)	2000
Protein (g)	100
Whole carbohydrate (g) 241 containing: 191 g maltodextrin (in control group) Or 161 g maltodextrin+30 g trehalose (in intervention group)	
Fat (g)	66
Fibre (g)	3
Vitamin A (mg)	242
Vitamin E (mg)	0.256
Vitamin K1 (µg)	0.039
Vitamin C (mg)	2.24
Vitamin B1 (mg)	0.778
Vitamin B9 (µg)	67
Calcium (mg)	572
Iron (mg)	5.9
Magnesium (mg)	88
Phosphorus (mg)	796
Zinc (mg)	4
Sodium (mg)	418
Potassium (mg)	1208

Manufacture of study supplements

Trehalose will be purchased from LeSen Biotechnology Co in trehalose hydrate form, with molecular formula of C12H22O11•2H2O, purity≥99% and low metal ion content.

Adverse event reporting and management

Self-limiting loose stools, flatulence and transient bloating may occur as minor to moderate gastrointestinal discomfort. Overall, these side effects are characteristic of disaccharide consumption.[41 42] In order to prevent, whole trehalose will not be presented in a single meal and nutritional and clinical conditions will be assessed daily. To manage feeding intolerance, one approach is transiently reduced the nutritional feeding, and another one is considering prokinetic agents if acute abdominal complications do not exitance.[40 43]

Study processes

This study is a phase II RCT, and the trial enrollment started on May 2021 and will be almost finished on August 2022. The study participants will be recruited from adult wards at trauma referral hospital, namely Shahid Kamyab in Mashhad, Iran. A summary of the registration schedule, supplementation and study evaluation are shown in figure 1.

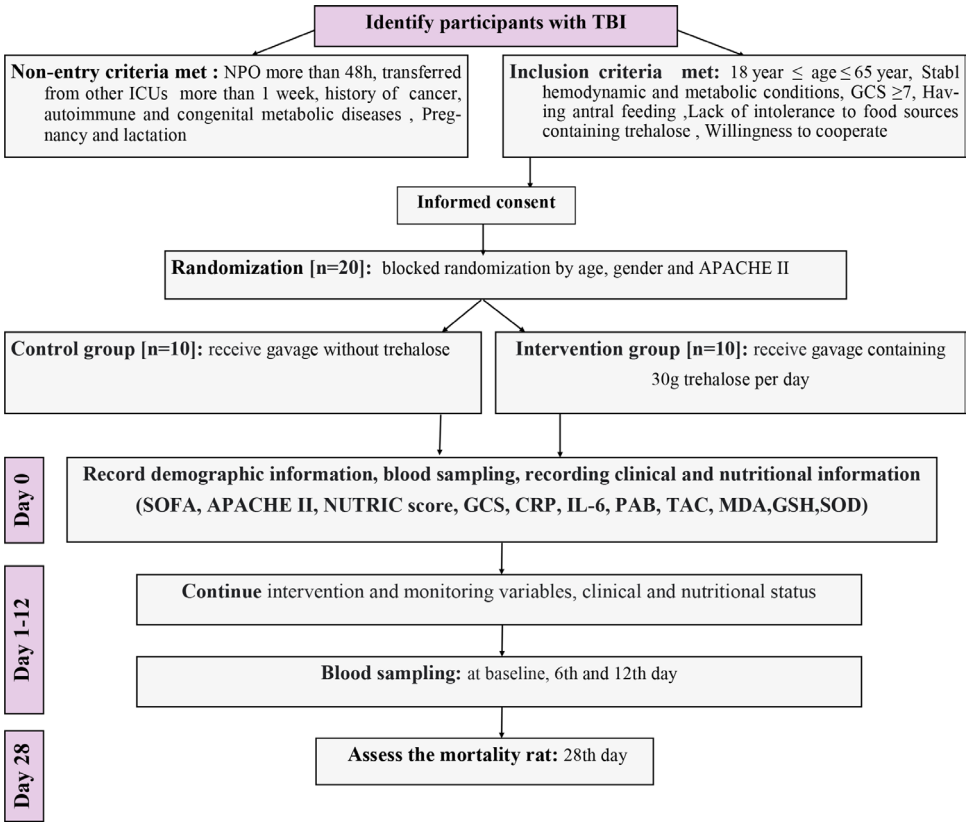

Figure 1 Trial protocol. APACHE II, Acute Physiology and Chronic Health Evaluation II; CRP, C reactive protein; GCS, Glasgow Coma Scale; GSH, glutathione; ICUs, intensive care units; IL-6, interleukin 6; MDA, malondialdehyde; NPO, nil per os; NUTRIC, Nutrition Risk in the Critically ill; PAB, pro-oxidant–antioxidant balance; SOD, superoxide dismutase; SOFA, Sequential Organ Failure Assessment; TAC, total antioxidant capacity; TBI, traumatic brain injury.

Outcomes

The primary efficacy outcomes of this study are to investigate the effect of 30 g trehalose consumption on the following criteria:

1. The changes in levels of inflammation will be measured by inflammatory marker 'IL-6' level and quantitative C reactive protein ('CRP').
2. The changes in oxidative stress level will be measured by pro-oxidant–antioxidant balance (PAB), total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione (GSH) and MDA.

Secondary outcomes of the study are:

1. Comparison of changes in GCS, APACHE II, NUTRIC and SOFA scores.
2. Comparison of blood glucose level, lipid profile and blood pressure.
3. Comparison of 28-day mortality.

Sample size

Because of the lack of a similar study in the patients specially in our target population, this study will be conducted as a pilot study. The sample size includes a total of 18 patients with 9 patients in each group.[44] To compensate for a drop-out rate of 10% during the study period, we increase the final sample size to 20 patients (10 patients in each group).

Study materials

Data will be recorded at these main times: at baseline, at the 6th day of intervention, at the 12th day of intervention and at the 28-day follow-up visit. Demographic information, anthropometric assessments including height (estimated by ulna length) at baseline, mid-arm circumference (with an extended arm, at mid-distance between the tip of the acromion and the olecranon), weight (by using a bed scale (Seca Medical 984)), body mass index and body composition (by using bioelectrical impedance analysis S10, InBody Company) will be measured at the beginning and the end of intervention.

The APACHE II Score and NUTRIC Score at baseline, 6th day, at the end of the study, SOFA Score and GCS at every day will be filled out. The nutritional requirement based on ESPEN guidelines will be evaluated individually.[40] Furthermore, 28 days by using the patient hospital electronic file or telephone call will be evaluated.

To evaluate inflammation and oxidative stress factors including IL-6, quantitative CRP, SOD, GSH, MDA, TAC the blood samples will be analysed by using ELISA kits. As well as, PAB that will be evaluated on a previously described method in a single assay.[45 46] A summary of schedule of interventions and assessments is shown in the table 2.

Table 2 Standard Protocol Items: Recommendations for Interventional Trials figure

	Enrolment	Allocation					
Timepoint	**Day 0**	**Day 0**	**Day 1**	**Day 3**	**Day 6**	**Day 12**	**Day 28**
Enrolment							
Eligibility screen	X						
Demographic data	X						
blood sample	X				x	X	
Allocation		X					
Interventions							
(Gavage containing trehalose)			←	—	→		
(Gavage without trehalose)			←	—	→		
Assessments							
CRP, IL-6, SOD, MDA, GSH, PAB, TAC			X		X	X	
SOFA, GCS			←	—	→		
APACHE II			X		X	X	
NUTRIC Score			X		X	X	
Blood sugar, blood pressure			←	—	→		
Lipid profile			X		X	X	
28-day mortality							X

Schedule of interventions and assessments.
APACHE II, Acute Physiologic Assessment and Chronic Health Evaluation II; CRP, C reactive protein; GCS, Glasgow Coma Scale/Score; GSH, glutathione; IL-6, interleukin 6; MDA, malondialdehyde; NUTRIC, Nutrition Risk in the Critically ill; PAB, pro-oxidant–antioxidant balance; SOD, superoxide dismutase; SOFA, Sequential Organ Failure Assessment; TAC, total antioxidant capacity.

In this method, the standard solutions are prepared by mixing varying proportions (0–100%) of 250 mM hydrogen peroxide with 3 mM uric acid in 10 mM sodium hydroxide. Overall, 60 mg tetramethylbenzidine (TMB) powder is dissolved in 10 mL dimethyl sulfoxide (DMSO) for preparation of TMB cation, 400 µL of TMB/DMSO is added in 20 mL of acetate buffer (0.05 M buffer, pH 4.5). Then, 70 µL of fresh chloramine T (100 mM) solution in distilled water is added into this 20 mL, mixed well, incubated for 2 hours at room temperature in a dark place. Overall, 25 U of peroxidase enzyme solution is added to 20 mL TMB cation, dispensed in 1 mL and stored at −20°C. To prepare the TMB solution, 200 mL of TMB/DMSO is added into 10 mL of acetate buffer (0.05 M buffer, pH 5.8). The working solution is prepared by mixing 1 mL TMB cation with 10 mL of TMB solution, incubated for 2 min at room temperature in a dark place and immediately used. A total of 10 µL of each sample, standard or blank, (distilled water) is mixed with 200 mL of working solution, plates, then incubated in a dark place at 37°C for 12 min. At the end of the incubation time, 100 mL of 2 M HCl is added to each well and measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. The standard curve is prepared by the values determined in standard samples. The values of the PAB assay are expressed in an arbitrary Hamidi-Koliakos unit based on the percentage of hydrogen peroxide evaluated in standard solution. Finally, the patient's PAB values are determined according to the prepared standard curve.

Blood samples collection

Overall, 10 cm^3 of the venous blood sample will be obtained from the patient at baseline, at 6th day and 12th day of the study. The samples will be transferred into a chelated gel tube and then kept at room temperature for 10 min, and then centrifuged in the laboratory of the hospital to separate the serum at 3600 rpm. Serum samples will be poured into 0.5 microtubes and stored at −20°C. Every 3 days, after collecting the samples, they will be transferred into the freezer at −78°C in the medical school for future inflammatory cytokine and oxidative stress factors measurements. Blood sugar and blood pressure will be checked daily and lipid profiles will be checked weekly in Hospitals Lab Mashhad, Iran. Serum IL-6, CRP, SOD, MDA, GSH, PAB and TAC will be analysed at the Immunology Lab of Bu Ali Research Institute, Mashhad, Iran.

Randomisation

Randomisation is performed by permuted block randomisation method, with block size equal to 4. The allocation ratio is 1:1. Using the site www.sealedenvelope.com, a random sequence of five blocks will be generated, each containing four patients.

Block randomisation method will be considered for this trial study. TBI patients will be randomised (in quadruple blocks) into the intervention and control group based on a blinded randomisation list generated by an online tool for clinical trials (https://www.sealedenvelope.com) and will be managed by the research director of the clinical nutrition department, Mashhad University of Medical Sciences.[47] The classification is based on age, gender and APACHE II Score (0–35 and 35–71) using quadruple blocks. The specific code for the participants and its treatment group is placed in different envelopes from 1 to 20. On the arrival of each eligible volunteer, informed consent will be obtained and then the first envelope will be opened, and their treatment group is determined. Allocation concealment will be considered using opaque-sealed sequentially numbered envelopes.

Blinding

In this study, participants and data analysts will be blinded. For proper blinding, the drug (enteral feeding containing trehalose) and placebo (enteral feeding without trehalose) will be precisely the same in colour, odour and packing. The analyst will be unaware of the intervention and control groups. In case of special clinical conditions and life-threatening side effects, unblinding will have occurred.

Statistical plan

Statistical methods for primary and secondary outcomes

The data will be analysed by SPSS V.16. Data gathering quality is checked via outlier analysis. Any suspected value will be checked with paper forms. However, no outliers would be removed. After this control, appropriate tables and graphs are used to describe the data. Quantitative variables that follow the normal distribution are presented as mean and SD, while quantitative variables that do not follow the normal distribution will be presented as median and quadratic amplitude. On the other hand, qualitative variables are displayed as frequency and percentage. The distribution of qualitative variables between and within the group will be analysed using the χ^2 test or Fisher exact test. For intergroup and intragroup comparisons of quantitative baseline variables with normal distribution, independent sample t-test and paired t-test are used, respectively. If the variables do not follow the normal distribution Mann-Whitney and Wilcoxon rank tests will be used, respectively. The significance level in tests is considered less than 0.05. To control the effect of possible confounders (ie, age, gender, GCS, food and drug intake and tolerance) covariance analysis will be used. The statistical analysis would be based on intention-to-treat method (ITT). At the end of the study, repeated measure one-way analysis of variance or multivariable covariance analysis will be conducted to examine differences in APACHE II Score, SOFA Score, severity of brain injury score (GCS) and all variables between the two groups. If the results differ after adjusting for the variables, both crude and adjusted findings will be reported. Since short duration of treatment may be informative about tolerance it will remain in the ITT analysis; whereas a secondary per protocol analysis with a minimal duration of the intervention for biochemical measurements is planned.

Patient and public involvement

Patients or the public have not been involved in the design, or conduct, or reporting of this trial.

DISCUSSION

The pathophysiology of TBI brain injury is divided into the primary brain injury and secondary brain injury. Based on previous studies, it has been shown that there is the pro-oxidant/antioxidant imbalance and increase in inflammatory factors in pathology of TBI patients.[48 49] Previous studies have demonstrated that trehalose might be involved in cellular autophagy.[21] In vivo and in vitro studies have shown that trehalose can inhibit inflammatory responses to endotoxic shock.[11 16 24] On the other hand, there is evidence that oral administration of this substance is safe up to 100 g.[42] The results of one of these animal studies suggested that using trehalose is a secure biophysics approach to adjusting inflammatory response in critically ill patients.[11] Therefore, this trial is designed as a pilot study. Due to the best of our knowledge, no previous studies have investigated the effect of oral trehalose consumption on inflammation and oxidative markers in TBI patients. After trehalose consumption, if a decrease in mortality, inflammation and oxidative markers, and improvement in nutritional and clinical status in TBI patients is observed, it would provide evidence for other clinical trials with a larger sample size. Finding a substance with therapeutic effects without toxicity is of great importance for TBI patients. We hope that oral trehalose will improve outcomes by these effects.

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Contributors MGD, AS and AN conceived the study. MGD, MS and HR contributed to the study design. MGD and FN drafted the first version of the manuscript. MK-R provided statistical expertise. The data collection and management, and writing of the report are the responsibility of MGD. All authors read and approved the final version of the manuscript, agreeing to be responsible for all aspects of the work.

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Patient consent for publication Consent obtained from parent(s)/guardian(s)

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