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Efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

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Title: Efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

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ABSTRACT

Introduction: Despite being one of the first diseases to be genetically characterised, β-thalassaemia remains a disorder without a cure in a majority. Most patients receive only supportive treatment therefore, have a poor quality of life and shorter life spans. Hydroxyurea is a drug that has shown to induce fetal haemoglobin synthesis and is recommended for the treatment of sickle cell disease. However, its usefulness in transfusion dependent β-thalassaemia is unclear. Here, we present a protocol for a randomized double-blind controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia.

Methods and analysis: This is a single-centre randomized double-blind placebo controlled clinical trial conducted at the Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka. Adult and adolescent patients with haematologically and genetically confirmed transfusion dependent β-thalassaemia are enrolled and randomised into the intervention or control group. The intervention group receives oral hydroxyurea 10-20mg/kg daily for six months while the control group receives a placebo which is identical in size, shape and colour to hydroxyurea without its active ingredient. Transfused blood volume, pre-transfusion haemoglobin, fetal haemoglobin level and adverse effects of treatment are monitored during treatment and for six months post-treatment. Cessation or reduction of blood transfusions during the treatment period will be the primary outcome measure. The statistical analysis will be based on intention to treat.

Ethics and dissemination: Ethical approval has been obtained from the Ethics Committee of Faculty of Medicine, University of Kelaniya (P/116/05/2018) and the trial is approved by the National Medicinal Regulatory Authority of Sri Lanka. Results of the trial will be disseminated in scientific publications in reputed journals.

Registration details: The trial is registered in the Sri Lanka Clinical Trials Registry (SLCTR/2018/024).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- A randomised double-blind placebo controlled clinical trial to evaluate the efficacy and safety of hydroxyurea in patients with transfusion dependent β-thalassaemia.
- Participants are genetically characterised for β-globin genotype, α-globin genotype, *XmnI* polymorphism and *BCL11A* polymorphism to identify predictors of response to hydroxyurea.
- The effects of hydroxyurea on multiple outcomes are evaluated; i.e. transfusion requirement, baseline haemoglobin, fetal haemoglobin level and ineffective erythropoiesis.
- Participants continue to receive transfusions according to standard protocols therefore, this study will not be able to determine the ability of hydroxyurea to maintain a safe but lower steady state haemoglobin level.

INTRODUCTION

β-Thalassaemia is one of the most common genetic diseases in the world(1). It is estimated that 70,000 children are born annually with β-thalassaemia worldwide(2). The most severely affected patients develop profound anaemia during infancy which is life-threatening without blood transfusions(3). The only existing cure is allogeneic haematopoietic stem cell transplantation that is available only to a small subset of patients with matched related donors(4). All other patients, an overwhelming majority, are managed conservatively with supportive treatment using regular blood transfusions and iron chelation for life(5, 6). Due to complications of the disease, these patients experience a poor quality of life and die prematurely in their fourth or fifth decade(7, 8).

The pathophysiology of β-thalassaemia centres around the unbalanced synthesis of α- and β-like globin chains in erythroid cells(9). α-Globin pairs with β-globin to form haemoglobin (Hb) A during postnatal life and γ-globin to form HbF during fetal life in healthy humans. In β-thalassaemia, the synthesis of normal β-globin is markedly reduced due to autosomal recessively inherited mutations of the β-globin gene(10). The resultant unbalanced synthesis and accumulation of α-globin chains lead to ineffective erythropoiesis and haemolysis causing severe anaemia(11). Several genetic modifiers which include augmentation of the synthesis of HbF by natural mutations that re-activate γ-globin are known to decrease α-globin excess and ameliorate the severity of β-thalassaemia(12).

Hydroxyurea is a cytostatic agent that interrupts DNA synthesis by inhibiting the ribonucleotide reductase pathway(13). It is a well-tolerated FDA approved oral

medication that is widely used in the treatment of cancers. However, it has been reported as a potent inducer of γ-globin in human erythroid cells in several pre-clinical studies(14, 15). It has also shown to be effective as a HbF inducing agent in patients with sickle cell disease and non-transfusion dependent β-thalassaemia(16-18). However, the benefit of hydroxyurea in patients with transfusion dependent β-thalassaemia is equivocal and has not been properly studied in randomised clinical trials. A recent Cochrane review and a meta-analysis that analysed the effect of hydroxyurea concluded that there is not enough evidence from randomized controlled trials to show hydroxyurea is effective in reducing transfusion requirements in patients with transfusion dependent thalassaemia(17, 19). These reviews recommended conducting well-designed randomized controlled trials to evaluate the same. In this paper, we present the protocol for a randomized double blind placebo controlled clinical trial to evaluates the efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia.

METHODS AND ANALYSIS

Study design and setting

This study is an ongoing single-centre, randomized, double-blind, placebo controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea for transfusion dependent β-thalassaemia. The study is conducted at the Adult and Adolescent Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka.

Study hypothesis

Oral hydroxyurea is an effective, tolerable and safe treatment that upregulates HbF production, improves pre-transfusion haemoglobin level and decreases transfusion requirement in patients with transfusion dependent β-thalassaemia.

Study population and eligibility criteria

Patients with haematologically and genetically confirmed transfusion dependent β-thalassemia attending Adult and Adolescent Thalassemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka will consist of the study population for this clinical trial.

Inclusion criteria:

- Patients with confirmed genotypes of homozygous/compound heterozygous β-thalassaemia major or HbE β-thalassaemia
- Patients who are aged over 12 years
- Patients who required more than 8 blood transfusions during the preceding 12 months

Exclusion criteria:

- Sickle β-thalassaemia
- Co-existing chronic liver disease
- Co-existing chronic kidney disease
- Co-existing viral hepatitis
- Patients with contraindications for hydroxyurea (eg: hypersensitivity, bone marrow depression, pregnancy and lactation)
- Patients who are expecting to get pregnant during the next 12 months
- Patients who have undergone bone marrow transplantation
- Patients on immunosuppressant therapy
- Baseline serum ferritin >5000ng/ml
- Baseline white cell count <4000/μL
- Baseline platelet count <150,000/μL
- Patients who have been started on regular transfusions for a pre-determined limited period

Sample size

The sample size was calculated based on an anticipated response rate of 26% in the hydroxyurea treatment group reported in previous observational studies, a type I error of 0.05 and a power of 80%(19). The calculated minimum sample size is 50 with 25 patients each in the intervention and control groups. Considering a 10% drop-out rate we aim to recruit 56 patients for the study.

Subject enrolment, randomisation and blinding

All eligible patients who fulfil inclusion criteria will be given a patient information sheet to read, given time to clarify doubts with investigators before obtaining informed written consent from them (Figure 1). When the patient is below the age of 18 years, consent will be obtained from one of the parents and assent will be obtained from the patient. At the time of enrolment, information on socio-demographic background, family history, past medical history and present medical problems will be gathered using an interviewer-administered questionnaire. Height and weight will be recorded, and abdominal examination will be done to assess hepatic and splenic sizes.

Then patients will be randomised into intervention or placebo group using a stratified block randomisation method. The intervention group will receive hydroxyurea while the control group will receive a placebo. Each patient will be given a trial number for identification. Hydroxyurea or placebo is packed in sealed envelopes with the trial number of the patient at a third-party location and handed over to the investigators. Participants, data collectors, outcome adjudicators and data analysts will be blinded regarding the treatment until the final analysis of data is available.

Intervention

Patients in the intervention group will receive oral hydroxyurea (manufactured by Cadila Healthcare Ltd, India) 10-20mg/kg daily for six months. Patients who weigh less than 50kg receives one 500mg capsule of hydroxyurea while those who weigh over 50kg will receive two 500mg capsules. Patients in the control group will receive the same number of capsules per body weight of a placebo which is identical in size, shape and colour to hydroxyurea. Placebo capsules contain ingredients identical to

hydroxyurea except for its active ingredient (manufactured by State Pharmaceutical Manufacturing Cooperation, Sri Lanka). All other standard treatment that includes blood transfusion and iron chelation will be continued. Patients will receive leuco-depleted packed red blood cell transfusions when haemoglobin drops below 9g/dl as per unit protocol which is in line with the International Thalassaemia Federation Guidelines(4).

Study procedure

Clinical evaluation

All patients will be reviewed at least monthly during the 6-month intervention period and followed-up for a further 6 months after discontinuation of treatment. During each review visit, a trained doctor will complete an interviewer-administered questionnaire to assess symptoms of anaemia, known and unknown adverse effects of hydroxyurea and tolerability of hydroxyurea. A complete physical examination will be done to assess weight, height, adverse effects of hydroxyurea and hepatic and splenic sizes. The exact volume of blood transfused during each visit will be recorded.

Laboratory evaluation

Full blood count, haemoglobin sub-type quantification, α- and β-globin genotyping and *Xmn1* and *BCL11A* polymorphism status will be performed at enrolment to determine the molecular characteristics of the study population. Additionally, baseline serum ferritin, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels will be measured at enrolment. During each follow-up visit, full blood count and quantification of HbF will be performed. Serum ferritin, creatinine, AST and ALT

will subsequently be checked at 3-monthly intervals. Soluble transferrin receptor levels will be assessed at the time of enrolment and the completion of the intervention period.

Full blood counts will be performed using Coulter Ac•T 5diff CP haematology analyser® whereas, quantification of haemoglobin sub-types and variants will be done by capillary electrophoresis using Capillarys 2 Flex Piercing® (Sebia) instrument. Serum ferritin, creatinine, AST and ALT will be measured in a clinically accredited laboratory using standard protocols. Soluble transferrin receptor levels will be measured by enzyme linked immunosorbent assay (ELIZA) using monoclonal antibodies and a commercially available ELIZA kit (R & D technologies). DNA will be extracted from cell pellet using QIAGEN DNA mini kit for α- and β-globin genotype assays. Amplification-refractory mutation system (ARMS) polymerase chain reaction (PCR) method will be used to detect known β-thalassaemia mutations by manipulating the primer sequence at the 3' end. Gap-PCR method will be used to detect two common deletional mutations ($\alpha^{-3.7}$and $\alpha^{-4.2}$) of the α-globin gene and the presence of excess α- globin genes using previously published protocols(20). *Xmn1* and *BCL11A* polymorphisms will be identified by restriction fragment length polymorphism method as per published protocols(21, 22).

Compliance evaluation

At each review visit, the number of capsules actually taken by patients will be recorded during the 6-month intervention period. The compliance for treatment will be assessed by dividing the actual number of tablets taken by the estimated number of tablets to be taken.

Safety evaluation

At follow-up visits during the intervention and 6-month post-intervention periods, patients will be interviewed by a trained doctor to monitor for known and unknown adverse effects of hydroxyurea treatment. Known adverse effects of hydroxyurea include; eczema, skin depigmentation, infection, fever, headache, nausea, vomiting, constipation, gastric discomfort, leg ulcers, mucositis, weight gain, asthma, neutropenia and thrombocytopenia. Both hematological and clinical toxicity will be monitored so that patients could be discontinued from the study if toxicities are observed. All study participants will be given contact details of investigators if they wish to clarify any doubts regarding the trial and to report suspected side effects at any point of the trial.

Outcome measures

Primary outcome

Cessation or reduction in the blood transfusion requirement during the treatment period will be the primary outcome measure.

Secondary outcomes

1. A rise in fetal haemoglobin percentage
2. Reduction in ineffective erythropoiesis as measured by elevated soluble transferrin receptor levels
3. Compliance to treatment
4. Safety and side effects of treatment

Exploratory outcomes

1. Effect of *Xmn1* polymorphism on response to treatment
2. Effect of *BCL11A* polymorphism on response to treatment

Statistical analysis

Data will be analysed using univariate and multivariate analysis by IBM SPSS statistics version 25. The analysis will be based on intention to treat and dropouts and discontinued patients will be included in the analysis.

Data management and monitoring

All completed anonymised questionnaires and laboratory reports will be stored in locked cupboards with the participant serial number written on individual files. Only the investigators will have access to hard copies. The electronic database will be maintained as a password-protected file. Material obtained from blood samples will be safely destroyed after completion of the study.

Ethical considerations

Investigators will not be involved in making management decisions (other than hydroxyurea treatment) which will be done by the clinical team caring for subjects. All decisions regarding blood transfusions will be taken by the clinical management team according to unit protocols. Patients who develop severe adverse events (haematological or clinical) related or unrelated to the treatment during the study period and those who are unable to tolerate hydroxyurea will be discontinued from the study. Suspected adverse events will be reported according to national guidelines. Participants will have the right to withdraw from the trail at any point without providing explanations.

Ethical approval for the study has been obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya (Ref. P/116/05/2018). The trial is registered at the Sri Lanka Clinical Trials Registry (Ref: SLCTR/2018/024) and has been approved by the National Medicinal Regulatory Authority of Sri Lanka.

Termination of the trial

The trial will be terminated if;

- New information regarding the safety or efficacy of hydroxyurea that indicates a change in the known risk/benefit profile become available, such that the risk/benefit is no longer acceptable for subjects participating in the trial
- Significant violation of good clinical practise that compromises the ability to achieve study objectives or compromises subject safety

Study status

The trial commenced in August 2019 according to the protocol of version 2.0, 28 May 2018 and is currently open for recruitment. We have recruited 18 patients for the trial so far.

DISCUSSION

Despite being one of the first genetic diseases to be characterised precisely at the molecular level, β-thalassaemia remains a life-limiting disorder without an effective cure(23). Several attempts exploring different pathways are underway to devices a cure however, most of these use advanced experimental technologies like gene therapy or genome editing(24-27). Hence, these therapies may not be available to a majority of patients who live in low- and middle-income countries(28).

In this trial, we aim to determine the efficacy and safety of hydroxyurea in minimising the transfusion requirement and improving the clinical outcome of β-thalassaemia. Hydroxyurea is an already FDA approved drug which is currently used for other indications including sickle cell anaemia and non-transfusion dependent β-thalassaemia(29). However, its efficacy in patients with transfusion-dependent β-thalassemia is inconclusive. This trial is designed to address this knowledge gap regarding the treatment of β-thalassaemia. All other previous studies which aim to evaluate the efficacy of hydroxyurea for transfusion dependent thalassaemia are either observational studies or trails without control arms(19, 30). Our study is probably the first randomised double-blind placebo-controlled clinical trial to evaluate the efficacy of hydroxyurea in transfusion dependent β-thalassaemia.

Previous use of hydroxyurea has suggested a starting dose of 10-15mg/kg/day and dose increments by 5mg/kg/day until a maximum tolerable dose of 35mg/kg/day is reached (31, 32). Because hydroxyurea is available as 500mg capsules, we utilised a fixed dose range of 10-20mg/kg/day for this study. After oral administration, hydroxyurea is rapidly absorbed by the gastrointestinal tract and a peak plasma

concentration is detected after 1-4 hours. Disproportionately high mean peak plasma concentration and area under the curve is associated with the increasing dose hence, once daily dosing of hydroxyurea is recommended(32).

The effects of hydroxyurea in thalassaemia could be multiple. Therefore, in the current study, we aim to evaluate the effects of hydroxyurea on HbF levels, transfusion requirement, pre-transfusion haemoglobin and ineffective erythropoiesis. Additionally, we will evaluate several factors, for example α- and β-globin genotypes and genetic polymorphisms in the *Xmn1* and *BCL11A* loci of the genome, which may be important in determining the response to hydroxyurea. This is particularly relevant as polymorphisms in *BCL11A* and *Xmn1* have been suggested to effectively predict the response to hydroxyurea in non-transfusion dependent β-thalassemia patients(33, 34). Therefore, through these approaches, we will be able to provide important mechanistic data on the action of hydroxyurea which is still incomplete.

Limitations

One important limitation of this trial is that these patients are on regular transfusion regimens. It was deemed unethical to stop transfusions in these patients for us to evaluate the full efficacy of hydroxyurea to see whether these patients could maintain a steady-state, safe albeit lower haemoglobin level of approximately 7-8g/dl without transfusions. All patients in the trial will continue to receive standard treatment with transfusions when the haemoglobin drops below 9g/dl. Therefore, we will only be able to evaluate the reduction in transfusion requirement of these patients as an outcome measure. However, this is acceptable as most clinical trials in patients with

transfusion-dependent thalassaemia have demonstrated reductions in transfusion burden rather than a complete cessation of transfusions(35).

FIGURE LEGENDS

Figure 1 – Study design and participant flow through the study

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Author contributions: AP and SM conceived the study. NY, NW, CM, AM, AP and SM contributed to the study design. NY drafted the manuscript and CM and SM finalized the manuscript. All authors assisted in developing the protocol and have read, reviewed, edited and approved the final manuscript.

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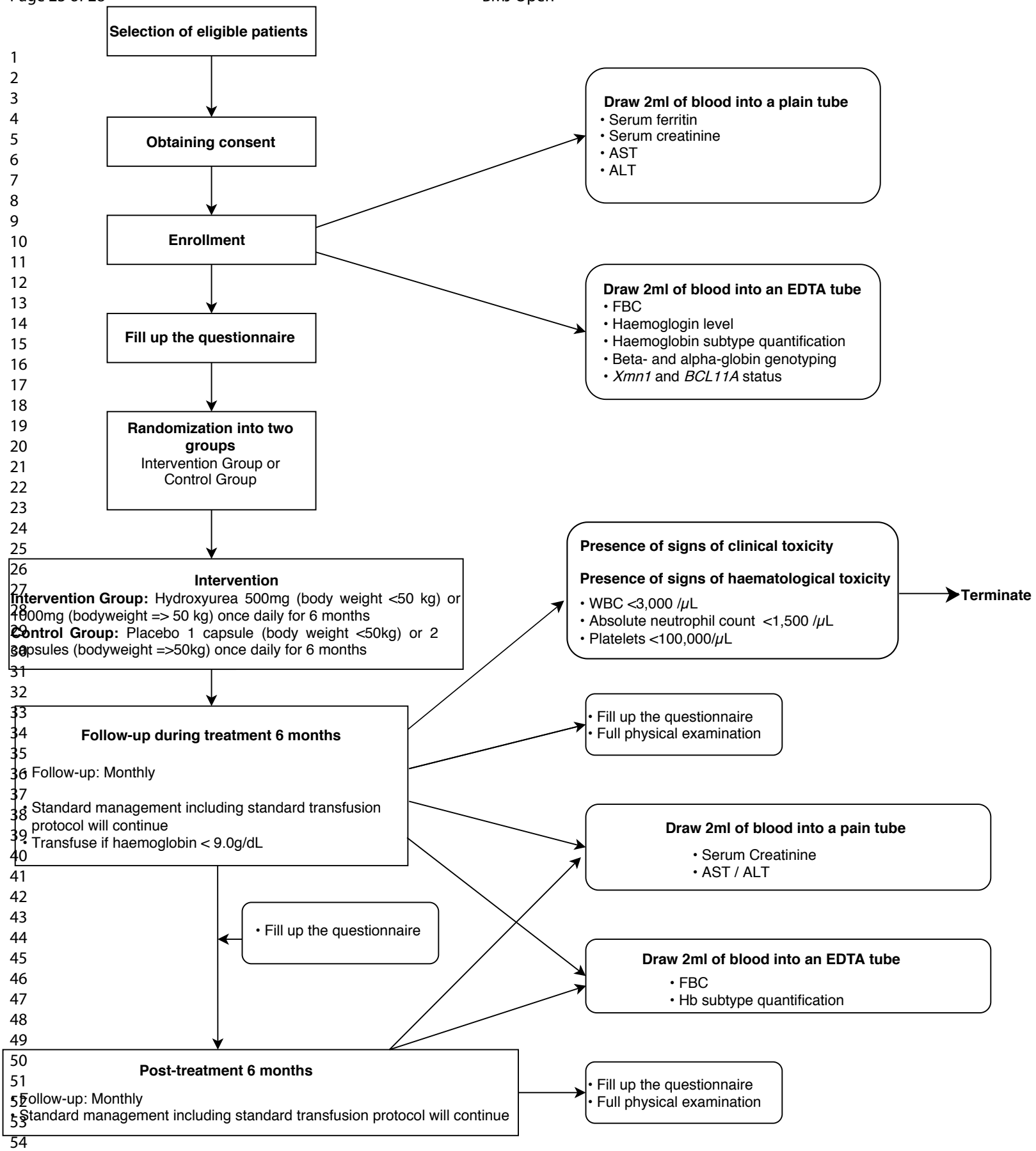
Patient and public involvement: It was not appropriate or possible to involve patients or the public in the design, conduct, reporting or dissemination plans of our research. Results of the investigations will be available for participants and will be used in the standard management when required.


Selection of eligible patients
Obtaining consent
Enrollment
Fill up the questionnaire
Randomization into two groups
Intervention Group or Control Group
Draw 2ml of blood into a plain tube
• Serum ferritin
• Serum creatinine
• AST
• ALT
Draw 2ml of blood into an EDTA tube
• FBC
• Haemoglogin level
• Haemoglobin subtype quantification
• Beta- and alpha-globin genotyping
• Xmn1 and BCL11A status
Intervention
Intervention Group: Hydroxyurea 500mg (body weight <50 kg) or 1000mg (bodyweight => 50 kg) once daily for 6 months
Control Group: Placebo 1 capsule (body weight <50kg) or 2 capsules (bodyweight =>50kg) once daily for 6 months
Presence of signs of clinical toxicity
Presence of signs of haematological toxicity
• WBC <3,000 /μL
• Absolute neutrophil count <1,500 /μL
• Platelets <100,000/μL
Terminate
Follow-up during treatment 6 months
• Follow-up: Monthly
• Standard management including standard transfusion protocol will continue
• Transfuse if haemoglobin < 9.0g/dL
• Fill up the questionnaire
• Full physical examination
Draw 2ml of blood into a pain tube
• Serum Creatinine
• AST / ALT
• Fill up the questionnaire
Draw 2ml of blood into an EDTA tube
• FBC
• Hb subtype quantification
Post-treatment 6 months
• Follow-up: Monthly
• Standard management including standard transfusion protocol will continue
• Fill up the questionnaire
• Full physical examination

Research protocol checklist

Manuscript title: Efficacy and safety of oral hydroxyurea for transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Included or not
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Yes
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Yes
	2b	All items from the World Health Organization Trial Registration Data Set	Yes
Protocol version	3	Date and version identifier	Yes
Funding	4	Sources and types of financial, material, and other support	Yes
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Yes
	5b	Name and contact information for the trial sponsor	Yes
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Yes
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Yes
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Yes
	6b	Explanation for choice of comparators	Yes
Objectives	7	Specific objectives or hypotheses	Yes
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Yes
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Yes
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Yes
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Yes
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Yes
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Yes
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Yes
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Yes

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Yes
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Yes
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Yes
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Yes
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Yes
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Yes
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Yes
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Yes
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Yes
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Yes
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Yes
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Yes
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Yes
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Yes
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Yes
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Yes

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Yes
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Yes
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Yes
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	Yes
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Yes
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Yes
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Yes
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Yes
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Yes
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Yes
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Yes

	31b	Authorship eligibility guidelines and any intended use of professional writers	Yes
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Yes
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Yes

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

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Title: Efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

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ABSTRACT

Introduction: Despite being one of the first diseases to be genetically characterised, β-thalassaemia remains a disorder without a cure in a majority. Most patients receive only supportive treatment therefore, have a poor quality of life and shorter life spans. Hydroxyurea is a drug that has shown to induce fetal haemoglobin synthesis and is recommended for the treatment of sickle cell disease. However, its usefulness in transfusion dependent β-thalassaemia is unclear. Here, we present a protocol for a randomised double-blind controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia.

Methods and analysis: This is a single-centre randomised double-blind placebo controlled clinical trial conducted at the Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka. Adult and adolescent patients with haematologically and genetically confirmed transfusion dependent β-thalassaemia are enrolled and randomised into the intervention or control group. The intervention group receives oral hydroxyurea 10-20mg/kg daily for six months while the control group receives a placebo which is identical in size, shape and colour to hydroxyurea without its active ingredient. Transfused blood volume, pre-transfusion haemoglobin, fetal haemoglobin level and adverse effects of treatment are monitored during treatment and for six months post-treatment. Cessation or reduction of blood transfusions during the treatment period will be the primary outcome measure. The statistical analysis will be based on intention to treat.

Ethics and dissemination: Ethical approval has been obtained from the Ethics Committee of Faculty of Medicine, University of Kelaniya (P/116/05/2018) and the trial is approved by the National Medicinal Regulatory Authority of Sri Lanka. Results of the trial will be disseminated in scientific publications in reputed journals.

Registration details: The trial is registered in the Sri Lanka Clinical Trials Registry (SLCTR/2018/024).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study evaluates the efficacy of oral hydroxyurea among patients with β-thalassaemia using a randomised double-blind placebo controlled clinical trial study design
- We will evaluate the genetic and clinical characteristics of study participants to identify predictors of response to hydroxyurea
- This study evaluate the effect of hydroxyurea on multiple outcomes
- As standard transfusion protocols are continued this study will not determine the ability of hydroxyurea to maintain a safe but lower steady state haemoglobin level
- As we use pretransfusion haemoglobin cut off of 9.0g/dL, this study will not accurately measure changes in fetal haemoglobin concentration

INTRODUCTION

β-Thalassaemia is one of the most common genetic diseases in the world[1]. It is estimated that 70,000 children are born annually with β-thalassaemia worldwide[2]. The most severely affected patients develop profound anaemia during infancy which is life-threatening without blood transfusions[3]. The only existing cure is allogeneic haematopoietic stem cell transplantation that is available only to a small subset of patients with matched related donors[4]. All other patients, an overwhelming majority, are managed conservatively with supportive treatment using regular blood transfusions and iron chelation for life[5 6]. Due to complications of the disease, these patients experience a poor quality of life and die prematurely in their fourth or fifth decade[7 8].

The pathophysiology of β-thalassaemia centres around the unbalanced synthesis of α- and β-like globin chains in erythroid cells[9]. α-Globin pairs with β-globin to form haemoglobin (Hb) A during postnatal life and γ-globin to form HbF during fetal life in healthy humans. In β-thalassaemia, the synthesis of normal β-globin is markedly reduced due to autosomal recessively inherited mutations of the β-globin gene[10]. The resultant unbalanced synthesis and accumulation of α-globin chains lead to ineffective erythropoiesis and haemolysis causing severe anaemia[11]. Several genetic modifiers which include augmentation of the synthesis of HbF by natural mutations that re-activate γ-globin are known to decrease α-globin excess and ameliorate the severity of β-thalassaemia[12].

Hydroxyurea is a cytostatic agent that interrupts DNA synthesis by inhibiting the ribonucleotide reductase pathway[13]. It is a well-tolerated FDA approved oral

medication that is widely used in the treatment of cancers. However, it has been reported as a potent inducer of γ-globin in human erythroid cells in several pre-clinical studies[14 15]. It has also shown to be effective as a HbF inducing agent in patients with sickle cell disease and non-transfusion dependent β-thalassaemia[16-18]. However, the benefit of hydroxyurea in patients with transfusion dependent β-thalassaemia is equivocal and has not been properly studied in randomised clinical trials[19]. Two recent Cochrane reviews that analysed the effects of hydroxyurea concluded that there is not enough evidence from randomized controlled trials to show hydroxyurea is effective in reducing transfusion requirements in patients with transfusion dependent thalassaemia[17 20]. These reviews recommended conducting well-designed randomised controlled trials to evaluate the same. In this paper, we present the protocol for a randomised double blind placebo controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia.

METHODS AND ANALYSIS

Study design and setting

This study is an ongoing single-centre, randomised, double-blind, placebo controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea for transfusion dependent β-thalassaemia. The study is conducted at the Adult and Adolescent Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka.

Study hypothesis

Oral hydroxyurea is an effective, tolerable and safe treatment that upregulates HbF production, improves pre-transfusion haemoglobin level and decreases transfusion requirement in patients with transfusion dependent β-thalassaemia.

Study population and eligibility criteria

Patients with haematologically and genetically confirmed transfusion dependent β-thalassaemia attending Adult and Adolescent Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka will consist of the study population for this clinical trial.

Inclusion criteria:

- Patients with confirmed genotypes of homozygous/compound heterozygous β-thalassaemia major or HbE β-thalassaemia
- Patients who are aged over 12 years
- Patients who required more than 8 blood transfusions during the preceding 12 months

Exclusion criteria:

- Sickle β-thalassaemia
- Co-existing chronic liver disease
- Co-existing chronic kidney disease
- Co-existing viral hepatitis
- Patients with contraindications for hydroxyurea (eg: hypersensitivity, bone marrow depression, pregnancy and lactation)
- Patients who are expecting to get pregnant during the next 12 months
- Patients who have undergone bone marrow transplantation
- Patients on immunosuppressant therapy
- Baseline serum ferritin >5000ng/mL
- Baseline white cell count <4000/μL
- Baseline platelet count <150,000/μL
- Patients who have been started on regular transfusions for a pre-determined limited period

Sample size

The sample size was calculated based on an anticipated response rate of 26% in the hydroxyurea treatment group reported in previous observational studies, a type I error of 0.05 and a power of 80%[19]. The calculated minimum sample size is 50 with 25 patients each in the intervention and control groups. Considering a 10% drop-out rate we aim to recruit 56 patients for the study.

Subject enrolment, randomisation and blinding

All eligible patients who fulfil inclusion criteria will be given a patient information sheet to read, given time to clarify doubts with investigators before obtaining informed written consent from them (Figure 1). When the patient is below the age of 18 years, consent will be obtained from one of the parents and assent will be obtained from the patient. At the time of enrolment, information on socio-demographic background, family history, past medical history and present medical problems will be gathered using an interviewer-administered questionnaire. Height and weight will be recorded, and abdominal examination will be done to assess hepatic and splenic sizes.

Then patients will be randomised into intervention or placebo group using a stratified block randomisation method. The intervention group will receive hydroxyurea while the control group will receive a placebo. Each patient will be given a trial number for identification. Hydroxyurea or placebo is packed in sealed envelopes with the trial number of the patient at a third-party location and handed over to the investigators. Participants, data collectors, outcome adjudicators and data analysts will be blinded regarding the treatment until the final analysis of data is available.

Intervention

Patients in the intervention group will receive oral hydroxyurea (manufactured by Cadila Healthcare Ltd, India) 10-20mg/kg daily for six months. Patients who weigh less than 50kg receives one 500mg capsule of hydroxyurea while those who weigh over 50kg will receive two 500mg capsules. Patients in the control group will receive the same number of capsules per body weight of a placebo which is identical in size, shape and colour to hydroxyurea. Placebo capsules contain ingredients identical to

hydroxyurea except for its active ingredient (manufactured by State Pharmaceutical Manufacturing Cooperation, Sri Lanka). All other standard treatment that includes blood transfusion and iron chelation will be continued. Patients will receive leuco-depleted packed red blood cell transfusions when haemoglobin drops below 9g/dL as per unit protocol which is in line with the International Thalassaemia Federation Guidelines[4].

Study procedure

Clinical evaluation

All patients will be reviewed at least monthly during the 6-month intervention period and followed-up for a further 6 months after discontinuation of treatment. During each review visit, a trained doctor will complete an interviewer-administered questionnaire to assess symptoms of anaemia, known and unknown adverse effects of hydroxyurea and tolerability of hydroxyurea. A complete physical examination will be done to assess weight, height, adverse effects of hydroxyurea and hepatic and splenic sizes. The exact volume of blood transfused during each visit will be recorded.

Laboratory evaluation

Full blood count, haemoglobin sub-type quantification, α- and β-globin genotyping and *Xmn1* and *BCL11A* polymorphism status will be performed at enrolment to determine the molecular characteristics of the study population. Additionally, baseline serum ferritin, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels will be measured at enrolment. During each follow-up visit, full blood count and quantification of HbF will be performed. Serum ferritin, creatinine, AST and ALT

will subsequently be checked at 3-monthly intervals. Soluble transferrin receptor levels will be assessed at the time of enrolment and the completion of the intervention period.

Full blood counts will be performed using Coulter Ac•T 5diff CP haematology analyser® whereas, quantification of haemoglobin sub-types and variants will be done by capillary electrophoresis using Capillarys 2 Flex Piercing® (Sebia) instrument. Serum ferritin, creatinine, AST and ALT will be measured in a clinically accredited laboratory using standard protocols. Soluble transferrin receptor levels will be measured by enzyme linked immunosorbent assay (ELIZA) using monoclonal antibodies and a commercially available ELIZA kit (R & D technologies). DNA will be extracted from cell pellet using QIAGEN DNA mini kit for α- and β-globin genotype assays. Amplification-refractory mutation system (ARMS) polymerase chain reaction (PCR) method will be used to detect known β-thalassaemia mutations by manipulating the primer sequence at the 3' end. Gap-PCR method will be used to detect two common deletional mutations ($\alpha^{-3.7}$and $\alpha^{-4.2}$) of the α-globin gene and the presence of excess α- globin genes using previously published protocols[21]. *Xmn1* and *BCL11A* polymorphisms will be identified by restriction fragment length polymorphism method as per published protocols[22 23].

Compliance evaluation

At each review visit, the number of capsules actually taken by patients will be recorded during the 6-month intervention period. The compliance for treatment will be assessed by dividing the actual number of tablets taken by the estimated number of tablets to be taken.

Safety evaluation

At follow-up visits during the intervention and 6-month post-intervention periods, patients will be interviewed by a trained doctor to monitor for known and unknown adverse effects of hydroxyurea treatment. Known adverse effects of hydroxyurea include; eczema, skin depigmentation, infection, fever, headache, nausea, vomiting, constipation, gastric discomfort, leg ulcers, mucositis, weight gain, asthma, neutropenia and thrombocytopenia. Both hematological and clinical toxicity will be monitored so that patients could be discontinued from the study if toxicities are observed. All study participants will be given contact details of investigators if they wish to clarify any doubts regarding the trial and to report suspected side effects at any point of the trial.

Outcome measures

Primary outcome

Cessation or reduction in the blood transfusion requirement during the treatment period will be the primary outcome measure.

Secondary outcomes

1. A rise in fetal haemoglobin percentage
2. Reduction in ineffective erythropoiesis as measured by elevated soluble transferrin receptor levels
3. Compliance to treatment
4. Safety and side effects of treatment

Exploratory outcomes

1. Effect of *Xmn1* polymorphism on response to treatment
2. Effect of *BCL11A* polymorphism on response to treatment

Statistical analysis

Data will be analysed using univariate and multivariate analysis by IBM SPSS statistics version 25. The analysis will be based on intention to treat and dropouts and discontinued patients will be included in the analysis.

Data management and monitoring

All completed anonymised questionnaires and laboratory reports will be stored in locked cupboards with the participant serial numbers written on individual files. Only the investigators will have access to hard copies. The electronic database will be maintained as a password-protected file. Material obtained from blood samples will be safely destroyed after completion of the study. A three-member independent data monitoring committee comprised of a paediatrician, a haematologist and a biostatistician has been appointed to make appropriate recommendations on participant safety and trial integrity.

Ethical considerations

Investigators will not be involved in making management decisions (other than hydroxyurea treatment) which will be done by the clinical team caring for subjects. All decisions regarding blood transfusions will be taken by the clinical management team according to unit protocols. Patients who develop severe adverse events (haematological or clinical) related or unrelated to the treatment during the study period and those who are unable to tolerate hydroxyurea will be discontinued from the

study. Suspected adverse events will be reported according to the national guidelines. Participants will have the right to withdraw from the trail at any point without providing explanations.

Ethical approval for the study has been obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka (Ref. P/116/05/2018). The trial is registered at the Sri Lanka Clinical Trials Registry (Ref: SLCTR/2018/024) and is approved by the National Medicinal Regulatory Authority of Sri Lanka.

Termination of the trial

The trial will be terminated if;

- New information regarding the safety or efficacy of hydroxyurea that indicates a change in the known risk/benefit profile become available, such that the risk/benefit is no longer acceptable for subjects participating in the trial
- Significant violation of good clinical practise that compromises the ability to achieve study objectives or compromises subject safety

Study status

The trial commenced in August 2019 according to the protocol of version 2.0, 28 May 2018 and is currently open for recruitment. We have recruited 18 patients for the trial so far.

Patient and public involvement

The research question related to this clinical trial was based on the positive feedback from patients with non-transfusion dependent β-thalassaemia who are already taking

oral hydroxyurea. Reports of the investigations done as part of the trial will be available to all participants and used in the standard management when required. The results of the study will be disseminated to study participants and all patients with β-thalassaemia in Sri Lanka using patient education lectures after completion of the trial.

DISCUSSION

Despite being one of the first genetic diseases to be characterised precisely at the molecular level, β-thalassaemia remains a life-limiting disorder without an effective cure[24]. Several attempts exploring different pathways are underway to device a cure however, most of these use advanced experimental technologies like gene therapy or genome editing[25-28]. Hence, these therapies may not be available to a majority of patients who live in low- and middle-income countries[29].

In this trial, we aim to determine the efficacy and safety of hydroxyurea in minimising the transfusion requirement and improving the clinical outcome of transfusion dependent β-thalassaemia. Hydroxyurea is an already FDA approved drug which is currently used for other indications including sickle cell anaemia and non-transfusion dependent β-thalassaemia[30]. However, its efficacy in patients with transfusion-dependent β-thalassemia is inconclusive. This trial is designed to address this knowledge gap regarding the treatment of β-thalassaemia. All other previous studies which aim to evaluate the efficacy of hydroxyurea for transfusion dependent thalassaemia are either observational studies or trials without control arms[19 31-34]. Our study is probably the first randomised double-blind placebo-controlled clinical trial to evaluate the efficacy of hydroxyurea in transfusion dependent β-thalassaemia.

Previous use of hydroxyurea has suggested a starting dose of 10-15mg/kg/day and dose increments by 5mg/kg/day until a maximum tolerable dose of 35mg/kg/day is reached [35 36]. Because hydroxyurea is available as 500mg capsules, we utilised a fixed dose range of 10-20mg/kg/day for this study. After oral administration, hydroxyurea is rapidly absorbed by the gastrointestinal tract and a peak plasma concentration is

detected after 1-4 hours. Disproportionately high mean peak plasma concentration and area under the curve is associated with the increasing dose hence, once daily dosing of hydroxyurea is recommended[36].

The effects of hydroxyurea in thalassaemia could be multiple. Therefore, in the current study, we aim to evaluate the effects of hydroxyurea on transfusion requirement, pre-transfusion haemoglobin, HbF levels and ineffective erythropoiesis. Additionally, we will evaluate several factors, for example α- and β-globin genotypes and genetic polymorphisms in the *Xmn1* and *BCL11A* loci of the genome, which may be important in determining the response to hydroxyurea. This is particularly relevant as polymorphisms in *BCL11A* and *Xmn1* have been suggested to effectively predict the response to hydroxyurea in non-transfusion dependent β-thalassemia patients[37 38]. Therefore, through these approaches, we will be able to provide important mechanistic data on the action of hydroxyurea which is still incomplete.

Limitations

One important limitation of this trial is that these patients are on regular transfusion regimens. It was deemed unethical to stop transfusions in these patients for us to evaluate the full efficacy of hydroxyurea to see whether these patients could maintain a steady-state, safe albeit lower haemoglobin level of approximately 7-8g/dL without transfusions. All patients in the trial will continue to receive standard treatment with transfusions when the haemoglobin drops below 9g/dL. Therefore, we will only be able to evaluate the reduction in transfusion requirement of these patients as an outcome measure. Similarly, we may not be able to fully evaluate the effects on HbF. However, this is acceptable as most clinical trials in patients with transfusion-dependent

thalassaemia have demonstrated reductions in transfusion burden rather than a complete cessation of transfusions[39].

FIGURE LEGENDS

Figure 1 – Study design and participant flow through the study

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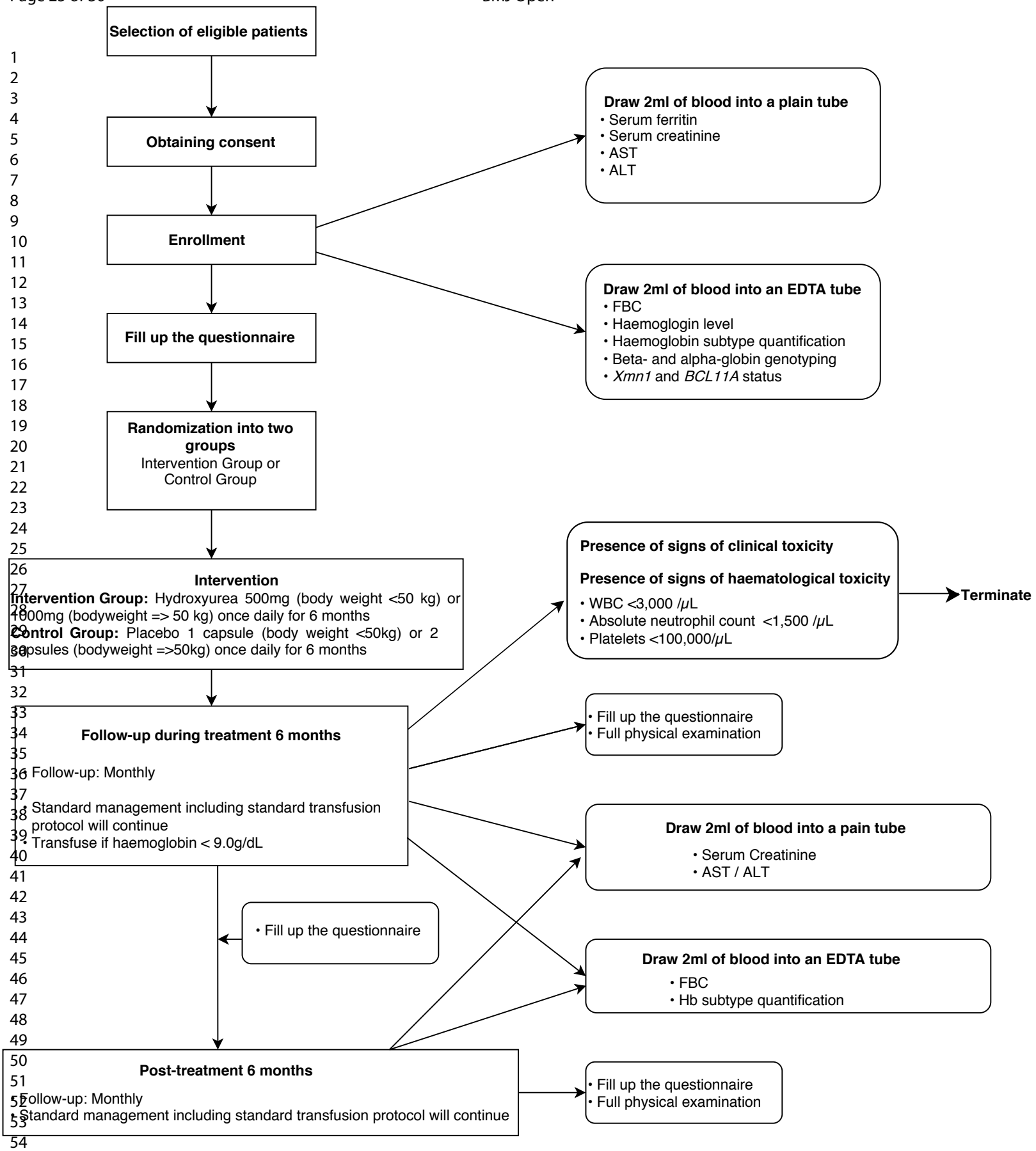
Selection of eligible patients
Obtaining consent
Enrollment
Fill up the questionnaire
Randomization into two groups
Intervention Group or Control Group
Draw 2ml of blood into a plain tube
• Serum ferritin
• Serum creatinine
• AST
• ALT
Draw 2ml of blood into an EDTA tube
• FBC
• Haemoglogin level
• Haemoglobin subtype quantification
• Beta- and alpha-globin genotyping
• Xmn1 and BCL11A status
Intervention
Intervention Group: Hydroxyurea 500mg (body weight <50 kg) or 1000mg (bodyweight => 50 kg) once daily for 6 months
Control Group: Placebo 1 capsule (body weight <50kg) or 2 capsules (bodyweight =>50kg) once daily for 6 months
Presence of signs of clinical toxicity
Presence of signs of haematological toxicity
• WBC <3,000 /μL
• Absolute neutrophil count <1,500 /μL
• Platelets <100,000/μL
Terminate
Follow-up during treatment 6 months
• Follow-up: Monthly
• Standard management including standard transfusion protocol will continue
• Transfuse if haemoglobin < 9.0g/dL
• Fill up the questionnaire
• Full physical examination
Draw 2ml of blood into a pain tube
• Serum Creatinine
• AST / ALT
• Fill up the questionnaire
Draw 2ml of blood into an EDTA tube
• FBC
• Hb subtype quantification
Post-treatment 6 months
• Follow-up: Monthly
• Standard management including standard transfusion protocol will continue
• Fill up the questionnaire
• Full physical examination

Research protocol checklist

Manuscript title: Efficacy and safety of oral hydroxyurea for transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page number/s
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	3, 14
Protocol version	3	Date and version identifier	14
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	23
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5,6
	6b	Explanation for choice of comparators	5,6
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7,8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11-12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12,13

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9-13, Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10-12
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12-14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13,14
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	13,14
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11-12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	3, 14, 15

	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10-11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

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Title: Efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

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ABSTRACT

Introduction: Despite being one of the first diseases to be genetically characterised, β-thalassaemia remains a disorder without a cure in a majority of patients. Most patients with β-thalassaemia receive only supportive treatment and therefore have a poor quality of life and shorter life spans. Hydroxyurea, which has shown to induce fetal haemoglobin synthesis in human erythroid cells is currently recommended for the treatment of sickle cell disease. However, its clinical usefulness in transfusion dependent β-thalassaemia is unclear. Here, we present a protocol for a randomised double-blind controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia.

Methods and analysis: This single-centre randomised double-blind placebo-controlled clinical trial is conducted at the Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka. Adult and adolescent patients with haematologically and genetically confirmed transfusion dependent β-thalassaemia are enrolled and randomised into the intervention or control group. The intervention group receives oral hydroxyurea 10-20mg/kg daily for six months while the control group receives a placebo which is identical in size, shape and colour to hydroxyurea without its active ingredient. Transfused blood volume, pre-transfusion haemoglobin level, fetal haemoglobin percentage and adverse effects of treatment are monitored during treatment and six months post-treatment. Cessation or reduction of blood transfusions during the treatment period will be the primary outcome measure. The statistical analysis will be based on intention to treat.

Ethics and dissemination: Ethical approval has been obtained from the Ethics Committee of Faculty of Medicine, University of Kelaniya (P/116/05/2018) and the trial is approved by the National Medicinal Regulatory Authority of Sri Lanka. Results of the trial will be disseminated in scientific publications in reputed journals.

Registration details: The trial is registered in the Sri Lanka Clinical Trials Registry (SLCTR/2018/024).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study evaluates the efficacy of oral hydroxyurea among patients with β-thalassaemia using a randomised double-blind placebo-controlled clinical trial study design
- We will evaluate the genetic and clinical characteristics of study participants to identify predictors of response to hydroxyurea treatment
- This study evaluates the effects of hydroxyurea on multiple outcomes
- As standard transfusion protocols are continued, this study will not determine the ability of hydroxyurea to maintain a safe but lower steady-state haemoglobin level
- As we use pretransfusion haemoglobin cut-off of 9.0g/dL, this study will not precisely measure changes in fetal haemoglobin percentage

INTRODUCTION

β-Thalassaemia is one of the most common genetic diseases in the world[1]. It is estimated that 70,000 children are born annually with β-thalassaemia worldwide[2]. The most severely affected patients develop profound anaemia during infancy, that is life-threatening without blood transfusions[3]. The only existing cure is allogeneic haematopoietic stem cell transplantation, which is available only to a small subset of patients with human leukocyte antigen matched sibling donors[4]. All other patients, an overwhelming majority, are managed conservatively with supportive treatment with regular blood transfusions and iron chelation for life[5 6]. Due to complications of the disease, these patients experience a poor quality of life and die prematurely in their fourth or fifth decade[7 8].

The pathophysiology of β-thalassaemia centres around the unbalanced synthesis of α- and β-like globin chains in erythroid cells[9]. In healthy humans, α-globin pairs with β- and γ-globin to form haemoglobin (Hb) A during postnatal life and HbF during fetal life, respectively. In β-thalassaemia, the synthesis of normal β-globin is markedly reduced due to autosomal recessively inherited mutations of the β-globin gene[10]. The resultant unbalanced synthesis and accumulation of α-globin chains lead to ineffective erythropoiesis and haemolysis, thus cause severe anaemia[11]. Several genetic modifiers, which include augmentation of the synthesis of HbF by natural mutations that re-activate γ-globin, are known to decrease α-globin excess and ameliorate the severity of β-thalassaemia[12].

Hydroxyurea is a cytostatic agent that interrupts DNA synthesis by inhibiting the ribonucleotide reductase pathway[13]. It is a well-tolerated FDA approved oral

medication that is widely used in the treatment of cancers. However, it has been reported as a potent inducer of γ-globin in human erythroid cells in several pre-clinical studies[14 15]. It has also shown to be effective as a HbF inducing agent in patients with sickle cell disease and non-transfusion dependent β-thalassaemia[16-18]. However, the benefit of hydroxyurea in patients with transfusion dependent β-thalassaemia is equivocal and has not been properly studied in randomised clinical trials[19]. Two recent Cochrane reviews that analysed the effects of hydroxyurea concluded, that the available evidence from clinical trials is insufficient to show hydroxyurea is effective in patients with transfusion dependent thalassaemia[17 20]. These reviews recommended conducting well-designed randomised controlled clinical trials to evaluate the effects of hydroxyurea in patients with transfusion dependent β-thalassaemia. In this paper, we present the protocol for a randomised double-blind placebo-controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea in transfusion dependent β-thalassaemia.

METHODS AND ANALYSIS

Study design and setting

This study is an ongoing single-centre, randomised, double-blind, placebo-controlled clinical trial to evaluate the efficacy and safety of oral hydroxyurea for transfusion dependent β-thalassaemia. The study is conducted at the Adult and Adolescent Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka.

Study hypothesis

Oral hydroxyurea is an effective, tolerable and safe treatment that upregulates HbF production, improves pre-transfusion haemoglobin level and decreases transfusion requirement in patients with transfusion dependent β-thalassaemia.

Study population and eligibility criteria

Patients with haematologically and genetically confirmed transfusion dependent β-thalassaemia attending Adult and Adolescent Thalassaemia Centre of Colombo North Teaching Hospital, Ragama, Sri Lanka will be the study population of this clinical trial.

Inclusion criteria:

- Patients with confirmed genotypes of homozygous / compound heterozygous β-thalassaemia major or HbE β-thalassaemia
- Patients who are aged over 12 years
- Patients who required more than eight blood transfusions during the preceding 12 months

Exclusion criteria:

- Sickle β-thalassaemia
- Co-existing chronic liver disease
- Co-existing chronic kidney disease
- Co-existing viral hepatitis
- Patients with contraindications for hydroxyurea (e.g., hypersensitivity, bone marrow depression, pregnancy and lactation)
- Patients who are expecting to get pregnant during the next 12 months
- Patients who have undergone bone marrow transplantation
- Patients on immunosuppressant therapy
- Baseline serum ferritin >5000ng/mL
- Baseline white cell count <4000/μL
- Baseline platelet count <150,000/μL
- Patients who were started on regular transfusions for a pre-determined limited period

Sample size

The sample size was calculated based on an anticipated response rate of 26% in the hydroxyurea treatment group reported in previous observational studies, a type I error of 0.05 and a power of 80%[19]. The calculated minimum sample size is 50 with 25 patients each in the intervention and control groups. Considering a 10% dropout rate, we aim to recruit 56 patients for the study.

Subject enrolment, randomisation and blinding

All eligible patients who fulfil inclusion criteria will be given a patient information sheet to read and time to clarify doubts with investigators before consenting. Informed written consent from all participants will be obtained before recruiting into the study (Figure 1). When the patient is below the age of 18 years, consent will be obtained from one of the parents, and assent will be obtained from the patient. At the time of enrolment, information on socio-demographic background, family history, past medical history and present medical problems will be gathered using an interviewer-administered questionnaire. Height and weight will be recorded, and abdominal examination will be done to assess hepatic and splenic sizes.

Then patients will be randomised into intervention or placebo group using a stratified block randomisation method. The intervention group will receive hydroxyurea while the control group will receive a placebo. Each patient will be given a trial number for identification. Hydroxyurea or placebo capsules are packed in sealed envelopes which are labelled with the trial number of each patient at a third-party location, and handed over to the investigators. Participants, data collectors, outcome adjudicators and data analysts will be blinded regarding the treatment until the final analysis of data is available.

Intervention

Patients in the intervention group will receive oral hydroxyurea (manufactured by Cadila Healthcare Ltd, India) 10-20mg/kg daily for six months. Patients who weigh less than 50kg receive one 500mg capsule of hydroxyurea while those who weigh over 50kg will receive two 500mg capsules. Patients in the control group will receive the

same number of capsules per body weight of a placebo which is identical in size, shape and colour to hydroxyurea. Placebo capsules (manufactured by State Pharmaceutical Manufacturing Cooperation, Sri Lanka) contain ingredients identical to hydroxyurea except for its active ingredient. All other standard treatments that include blood transfusions and iron chelation will be continued. Patients will receive leuco-depleted packed red blood cell transfusions when haemoglobin drops below 9g/dL as per unit protocol which is in line with the International Thalassaemia Federation Guidelines[4].

Study procedure

Clinical evaluation

All patients will be reviewed at least monthly during the 6-month intervention period and followed-up for a further six months after discontinuation of treatment. During each review visit, a trained doctor will complete an interviewer-administered questionnaire to assess symptoms of anaemia, known and unknown adverse effects of hydroxyurea and tolerability of hydroxyurea. A complete physical examination will be done to assess weight, height, adverse effects of hydroxyurea and hepatic and splenic sizes. The exact volume of transfused blood during each visit will be recorded.

Laboratory evaluation

Full blood count, haemoglobin sub-type quantification, α- and β-globin genotyping and *Xmn1* and *BCL11A* polymorphism status will be performed at enrolment to determine the molecular characteristics of the study population. Additionally, baseline serum ferritin, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels will be measured at enrolment. During each follow-up visit, full blood count

and quantification of HbF will be performed. Serum ferritin, creatinine, AST and ALT will subsequently be checked at 3-monthly intervals. Soluble transferrin receptor levels will be assessed at the time of enrolment and at the completion of the intervention period.

Full blood counts will be performed using Coulter Ac•T 5diff CP haematology analyser® whereas, quantification of haemoglobin sub-types and variants will be done by capillary electrophoresis using Capillarys 2 Flex Piercing® (Sebia) instrument. Serum ferritin, creatinine, AST and ALT will be measured in a clinically accredited laboratory using standard protocols. Soluble transferrin receptor levels will be measured by enzyme linked immunosorbent assay (ELIZA) using monoclonal antibodies and a commercially available ELIZA kit (R & D technologies). DNA will be extracted from the cell pellet using QIAGEN DNA mini kit for α- and β-globin genotype assays. Amplification-refractory mutation system (ARMS) polymerase chain reaction (PCR) method will be used to detect known β-thalassaemia mutations by manipulating the primer sequence at the 3' end. Gap-PCR method will be used to detect two common deletional mutations (α-$^{3.7}$and α-$^{4.2}$) of the α-globin gene and the presence of excess α-globin genes using previously published protocols[21]. *Xmn1* and *BCL11A* polymorphisms will be identified by restriction fragment length polymorphism method as per published protocols[22 23].

Compliance evaluation

At each review visit, the number of capsules actually taken by patients will be recorded during the 6-month intervention period. Treatment compliance will be assessed by

dividing the actual number of tablets taken by the estimated number of tablets to be taken.

Safety evaluation

At follow-up visits during the intervention and 6-month post-intervention periods, patients will be interviewed by a trained doctor to monitor for known and unknown adverse effects of hydroxyurea treatment. Known adverse effects of hydroxyurea include; eczema, skin depigmentation, infection, fever, headache, nausea, vomiting, constipation, gastric discomfort, leg ulcers, mucositis, weight gain, asthma, neutropenia and thrombocytopenia. Both haematological and clinical toxicity will be monitored so that patients could be discontinued from the study if toxicity develops. All study participants will be given contact details of investigators if they wish to clarify any doubts regarding the trial, and to report suspected adverse effects at any point of the trial.

Outcome measures

Primary outcome

Cessation or reduction of blood transfusion requirement during the treatment period will be the primary outcome measure.

Secondary outcomes

1. A rise in fetal haemoglobin percentage
2. Reduction in ineffective erythropoiesis as measured by elevated soluble transferrin receptor levels
3. Compliance to treatment

4. Safety and adverse effects of treatment

Exploratory outcomes

1. Effect of *Xmn1* polymorphism on response to treatment
2. Effect of *BCL11A* polymorphism on response to treatment

Statistical analysis

Data will be analysed using univariate and multivariate analysis by IBM SPSS statistics version 25. The analysis will be based on intention to treat, and dropouts and discontinued patients will be included in the analysis.

Data management and monitoring

All completed anonymised questionnaires and laboratory reports will be stored in locked cupboards with participant serial numbers written on individual files. Only the investigators will have access to hard copies. The electronic database will be maintained as a password-protected file. Material obtained from blood samples will be safely destroyed after completion of the study. A three-member independent data monitoring committee comprised of a paediatrician, a haematologist and a biostatistician has been appointed to make appropriate recommendations on participant safety and trial integrity.

Ethical considerations

Investigators will not be involved in making management decisions (other than hydroxyurea treatment) which will be done by the clinical team caring for subjects. All decisions regarding blood transfusions will be taken by the clinical management team

according to unit protocols. Patients who develop severe adverse events (haematological or clinical) related or unrelated to the treatment during the study period and those who are unable to tolerate hydroxyurea will be discontinued from the study. Suspected adverse events will be reported according to the national guidelines. Participants will have the right to withdraw from the trail at any point without providing explanations.

Ethical approval for the study has been obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka (Ref. P/116/05/2018). The trial is registered at the Sri Lanka Clinical Trials Registry (Ref: SLCTR/2018/024) and is approved by the National Medicinal Regulatory Authority of Sri Lanka.

Termination of the trial

The trial will be terminated if;

- New information regarding the safety or efficacy of hydroxyurea that indicates a change in the known risk/benefit profile becomes available, such that the risk/benefit is no longer acceptable for subjects participating in the trial
- Significant violation of good clinical practise that compromises the ability to achieve study objectives or compromises subject safety

Study status

The trial commenced in August 2019 according to the protocol of version 2.0, 28 May 2018 and is currently open for recruitment. We have recruited 18 patients for the trial so far.

Patient and public involvement

The research question related to this clinical trial was based on the positive feedback from patients with non-transfusion dependent β-thalassaemia who are already taking oral hydroxyurea. Reports of the investigations done as part of the trial will be available to all participants and used in the standard management when required. The results of the study will be disseminated to study participants and all patients with β-thalassaemia in Sri Lanka using patient education lectures after completion of the trial.

DISCUSSION

Despite being one of the first genetic diseases to be characterised precisely at the molecular level, β-thalassaemia remains a life-limiting disorder without an effective cure[24]. Nonetheless, several attempts exploring novel pathways are currently underway to develop a cure for β-thalassaemia. Most of these emerging therapies use advanced experimental technologies like gene therapy or genome editing[25-28], hence may not be available to a majority of patients living in low- and middle-income countries[29].

In this clinical trial, we aim to determine the efficacy and safety of oral hydroxyurea in minimising transfusion requirements and improving clinical outcomes of patients with transfusion dependent β-thalassaemia. Hydroxyurea is an already FDA approved drug which is currently being used for other indications including sickle cell disease and non-transfusion dependent β-thalassaemia[30]. However, its efficacy in patients with transfusion-dependent β-thalassemia is inconclusive. Our trial is designed to address this knowledge gap regarding treatment of β-thalassaemia. All previous studies which aim to evaluate the efficacy of hydroxyurea for transfusion dependent β-thalassaemia are either observational studies or trials without control arms[19 31-34]. Our study is probably the first randomised double-blind placebo-controlled clinical trial to evaluate the efficacy of hydroxyurea in transfusion dependent β-thalassaemia.

Previous use of hydroxyurea has suggested a starting dose of 10-15mg/kg/day and dose increments by 5mg/kg/day until a maximum tolerable dose of 35mg/kg/day is reached[35 36]. Because hydroxyurea is available as 500mg capsules, we utilised a fixed-dose range of 10-20mg/kg/day for this study. After oral administration,

hydroxyurea is rapidly absorbed by the gastrointestinal tract, and a peak plasma concentration is detected after 1-4 hours. Disproportionately high peak plasma concentration is associated with increasing dose; hence, once-daily dosing of hydroxyurea is recommended[36].

The effects of hydroxyurea in β-thalassaemia could be multiple. Therefore, in the current study, we aim to evaluate the effects of hydroxyurea on transfusion requirement, pre-transfusion haemoglobin, HbF levels and ineffective erythropoiesis. Additionally, we will evaluate several factors, for example α- and β-globin genotypes and genetic polymorphisms in the *Xmn1* and *BCL11A* genomic loci, which may be important in determining the response to hydroxyurea. This is particularly relevant as polymorphisms in *BCL11A* and *Xmn1* have been suggested to predict the response to hydroxyurea in non-transfusion dependent β-thalassemia patients[37 38]. Therefore, through these approaches, we will be able to provide important mechanistic data on the action of hydroxyurea which is still incomplete.

Limitations

One important limitation of this trial is that these patients are on regular transfusion regimens. It was deemed unethical to stop transfusions in these patients for us to evaluate the full efficacy of hydroxyurea to see whether these patients could maintain a steady-state, safe albeit lower haemoglobin level of approximately 7-8g/dL without transfusions. All patients in the trial will continue to receive standard treatment with transfusions when their haemoglobin drops below 9g/dL. Therefore, we will only be able to evaluate the reduction in transfusion requirement as an outcome measure. Similarly, we may not be able to evaluate the effects on HbF fully. However, this is

acceptable as most previous clinical trials on transfusion-dependent β-thalassaemia have demonstrated reduction in transfusion burden rather than complete cessation of transfusions[39].

FIGURE LEGENDS

Figure 1 – Study design and participant flow through the study

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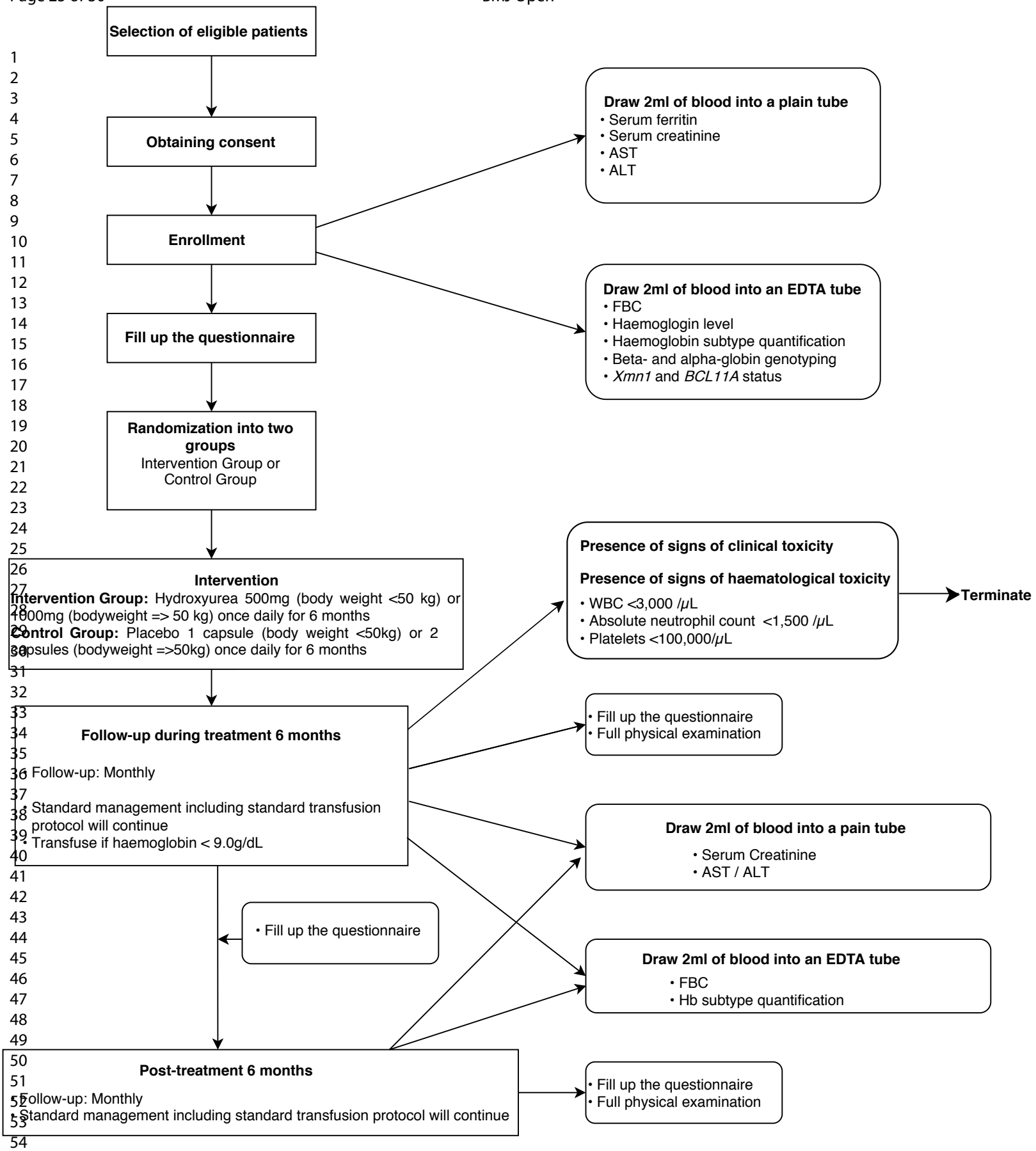
Competing interests statement: None declared.


Selection of eligible patients
Obtaining consent
Enrollment
Fill up the questionnaire
Randomization into two groups
Intervention Group or Control Group
Draw 2ml of blood into a plain tube
• Serum ferritin
• Serum creatinine
• AST
• ALT
Draw 2ml of blood into an EDTA tube
• FBC
• Haemoglogin level
• Haemoglobin subtype quantification
• Beta- and alpha-globin genotyping
• Xmn1 and BCL11A status
Intervention
Intervention Group: Hydroxyurea 500mg (body weight <50 kg) or 1000mg (bodyweight => 50 kg) once daily for 6 months
Control Group: Placebo 1 capsule (body weight <50kg) or 2 capsules (bodyweight =>50kg) once daily for 6 months
Presence of signs of clinical toxicity
Presence of signs of haematological toxicity
• WBC <3,000 /μL
• Absolute neutrophil count <1,500 /μL
• Platelets <100,000/μL
Terminate
Follow-up during treatment 6 months
• Follow-up: Monthly
• Standard management including standard transfusion protocol will continue
• Transfuse if haemoglobin < 9.0g/dL
• Fill up the questionnaire
• Full physical examination
Draw 2ml of blood into a pain tube
• Serum Creatinine
• AST / ALT
• Fill up the questionnaire
Draw 2ml of blood into an EDTA tube
• FBC
• Hb subtype quantification
Post-treatment 6 months
• Follow-up: Monthly
• Standard management including standard transfusion protocol will continue
• Fill up the questionnaire
• Full physical examination

Research protocol checklist

Manuscript title: Efficacy and safety of oral hydroxyurea for transfusion dependent β-thalassaemia: a protocol for randomised double-blind controlled clinical trial

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page number/s
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	3, 14
Protocol version	3	Date and version identifier	14
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	23
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	13
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5,6
	6b	Explanation for choice of comparators	5,6
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7,8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11-12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	13
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10-12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12,13

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9-13, Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10-12
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12-14
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13,14
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	13,14
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11-12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	3, 14, 15

	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10-11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.