Efficacy and Safety of High-Dose Vitamin D Supplementation for Overactive Bladder Dry in Children: A Randomized Clinical Trial

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1. STUDY OBJECTIVES

The purpose of this study was to assess the clinical efficacy and safety of high-dose Vitamin D Supplementation (VDS) in combination with Standard Urotherapy (SU) for the treatment of Overactive Bladder (OAB) dry among pediatric patients, as compared to (1) solifenacin, a commonly utilized anticholinergic medication, combined with SU, and (2) SU alone. This study endeavors to contribute to the current understanding of the optimal treatment approach for managing OAB dry in this population and has the potential to inform clinical decision-making and improve patient outcomes.

2. BACKGROUND AND RATIONALE

According to the International Children's Continence Society (ICCS), OAB is a syndrome that manifests as urinary urgency, frequently accompanied by frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or any other obvious pathology[1]. This common and concerning condition can affect individuals of all ages[2]. Approximately, OAB has been identified with a prevalence of 5-12% in children aged 5-10 years and 0.5% in older adolescents aged 16-18 years[1]. OAB may manifest as either OAB-wet, which is characterized by urgency urinary incontinence, or OAB-dry, which is characterized by urgency without

incontinence[2]. Furthermore, it is noteworthy that those with OAB, even without urine loss, experience a reduced quality of life (QoL) compared to those without this condition[3]. Studies on symptoms related to OAB have revealed that they can significantly impact patients' QoL, with OAB-dry being just as distressing as urinary incontinence. Despite extensive research, the underlying pathological mechanism is not yet fully understood, and its complex etiology has resulted in a variety of treatment options[4]. While behavioral and drug therapies are the primary treatments for OAB, they may not be fully effective in curing the condition for all patients.

The first-line treatment for OAB is SU, which comprises several essential components. These include providing patients with information and demystifying their condition, instructing them on how to resolve lower urinary tract (LUT) dysfunction, offering lifestyle advice, registering symptoms and voiding habits, and providing ongoing support and encouragement through regular follow-up sessions with the caregiver[5]. Anticholinergic medications, also known as antimuscarinics, are commonly prescribed as the first-line treatment for OAB in children who exhibit persistent symptoms despite undergoing SU. Solifenacin succinate is a medication that falls into this category and is primarily employed to alleviate symptoms of OAB, such as urinary urgency, frequency, and incontinence. Although solifenacin succinate has demonstrated efficacy in clinical studies, it may result in common adverse effects, including xerostomia, constipation, blurred vision, and headache[6,7]. These side effects may pose a challenge to patients' adherence to medication, particularly during prolonged

treatment.

There are two distinct forms of vitamin D in nature: vitamin D_3 of animal origin (cholecalciferol) and vitamin D_2 of plant origin (ergocalciferol). In humans, vitamin D_3 is produced in the skin through exposure to sunlight, whereas vitamin D_2 and D_3 can be obtained from the diet, though only a small number of foods contain substantial amounts of the vitamin[8,9]. Vitamin D_2 and D_3 are conveyed via vitamin D-binding proteins to the liver, where they are hydroxylated by vitamin D-25-hydroxylase (CYP2R1) to produce 25-hydroxyvitamin D [25(OH)D], the primary circulating vitamin D metabolite used to assess an individual's vitamin D status. 25(OH)D is then transported to the kidney, where it is hydroxylated by 25(OH)-D-1α-hydroxylase (CYP27B1) and further hydroxylated to 1,25-dihydroxy vitamin D [$1,25(OH)_2D$ or calcitriol], the biologically active hormonal form of vitamin D[10,11]. In addition to its well-established roles in bone metabolism and immune function, emerging research suggests that vitamin D status may be inversely associated with the incidence of several diseases[12-14]. The vitamin D receptor has been found in cell types not involved in bone metabolism, revealing its impact on diverse physiological systems[15,16]. These findings have prompted an increase in the number of studies investigating the potential benefits of VDS, both alone and in combination with other therapies, for a variety of medical conditions. Regardless of age and geographic location, a substantial number of patients have been observed to have deficient levels of this essential micronutrient[17]. Evidence suggests that there is an association between vitamin D deficiency and LUTS, such as

those associated with OAB disorder[18]. This finding opens up new possibilities for the management of OAB through VDS. Previous studies have reported that patients with OAB have lower serum vitamin D levels, and that VDS can serve as an alternative treatment option that reduces OAB-related symptoms and improves QoL in adults[19-21]. According to a meta-analysis and systematic review, high-dose VDS was linked with few supplement-related clinical adverse events in children between 0 to 6 years of age[22]. These findings suggest that VDS may be well-tolerated in young children within the dose range of 1200 to 10,000 IU/d.

3. STUDY DESIGN.

This study is conducted in the form of a prospective, observer-blind, parallel-group randomized controlled clinical trial. The study population will consist of children aged five years or older diagnosed with OAB-dry at the Children's Hospital of Chongqing Medical University, with serum vitamin D levels below 35ng/ml. We will exclude patients with other systemic organic diseases and those with poor compliance, thus representing a relatively ideal cohort. Participants will be randomly assigned to one of three intervention groups, including a group receiving a combination of high-dose vitamin D and SU, a group receiving a combination of solifenacin and SU, and a group receiving SU alone.

This design allows for a thorough evaluation of the comparative efficacy and safety of various treatment regimens while minimizing sources of bias and confusion.

The randomized controlled design provides confidence that any observed differences between groups can be directly attributed to the experimental intervention, while the observer-blind method helps eliminate observer bias in assessment and outcome evaluation.

The parallel-group design facilitates direct comparisons between the three intervention groups, generating insightful information about the relative efficacy of each regimen. The combination of high-dose vitamin D and SU represents a novel therapeutic approach. The solifenacin and SU combination group is included to investigate the effects of previously described anticholinergics agents on outcomes. The control group receiving SU alone serves as an important reference point, providing a baseline for comparisons with other intervention measures.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS.

4.1 Inclusion Criteria

- Children older than 5 years of age with a diagnosis of OAB dry (do not experience incontinence yet suffer from symptoms of urgency, frequency, and nocturia) seen at the outpatient urology clinics (diagnosis follows the latest International Children's Continence Society recommendations).

- Children with a serum vitamin D level of less than 35 ng/ml as indicated by the laboratory result.

- Written informed consent was obtained from the participants and their respective guardians.

4.2 Exclusion Criteria

- Those with a comorbidity of other urological abnormalities or serious diseases (e.g. hypospadias, cryptorchidism, posterior urethral valves, vesicoureteral reflux, neurogenic bladder, urological tumours, urinary stones, bladder and urethral injuries, etc.).

- Those with a comorbidity of neurological disorders (e.g. epilepsy, spinal cord injury, spinal cord dysplasia, spinal cord embolism syndrome, multiple sclerosis, autism spectrum disorder, etc.).

- Those with a comorbidity of severe heart disease, abnormal liver or kidney function, lung disease, skeletal deformities, severe gastrointestinal disease, or inherited metabolic disorders.

- Those with a history of gastrointestinal surgery and urinary tract surgery.

- Those with chronic constipation.

- Those taking anticonvulsant and antiepileptic drugs, hormones, anti-tuberculosis drugs.

- Those have a previous history of hypercalcemia, hyperphosphatemia with renal rickets.

- Those have a history of hematuria and urinary tract infection within the last 1 year.

- Those have a history of allergy or allergic disease to vitamin D preparations.

- Those Participate in other clinical studies at the time of consultation or during the follow-up of other clinical studies.

- Any condition that could cause urinary symptoms or interfere with assessment of efficacy parameters.

- Those unwilling to participate in this study.

4.3 Study Enrollment Procedures

Eligible participants will be selected from patients visiting the pediatric urology clinics at the Children's Hospital of Chongqing Medical University who have been diagnosed with OAB-dry and have serum vitamin D levels below 35ng/ml. The screening log will record reasons for both ineligibility and non-participation of eligible candidates. Prior to the commencement of intervention measures, interested patients will be required to provide informed consent.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

During the eight-week treatment period, the study was divided into three intervention groups, and each patient received follow-up care from an outpatient

physician every four weeks. Participants in each intervention group will be required to attend a 30-minute SU program per session.

Children assigned to the VDS+SU group will receive high-dose VDS (800 IU, three times per day) in addition to the biweekly SU course.

Children in the SOL+SU group will receive treatment with solifenacin succinate (5 mg once daily, with a maximum dose of 10 mg per day), in addition to the SU course.

Participants in the SU group only attend regular SU meetings, which provide basic information and an explanation of the disease, guidance on behavioral changes and lifestyle advice, registration of urinary habits using a bladder diary, as well as support and encouragement from caregivers.

5.2 Adherence Assessment

Upon enrollment, all participants will be required to complete all relevant intervention treatments, as well as subsequent questionnaire surveys and efficacy assessments, without any interruption to the intervention treatment.

6. STUDY PROCEDURES.

6.1 Schedule of Evaluations

Assessment	Enrollment: Day1	Run-in period (Day2-8)	Week 4 (Day 33-39)	Week 8 (Day 61-67)
Informed Consent Form	√			

Demographics	√			
Medical History	√		√	√
SU	√		√	√
Bladder Diary		√	√	√
Current Medications		√	√	√
measurements of biochemistry.	√			√
measurements of hematology,	√			√
measurements of urinalysis.	√			√
Adverse Events			√	√

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Eligible participants will be identified at the Outpatient Department of Urology, Children's Hospital of Chongqing Medical University. Patients will be further screened for eligibility by answering screening questions in a questionnaire (i.e., excluding exclusion criteria).

6.2.1.1 Consent Procedure

An informed consent form that describes the screening and study procedures will be used. The study coordinator will conduct the consent procedure in person. The

coordinator will describe the study to potential participants. The study coordinator and the principal investigator will regularly review the informed consent form for potential modifications.

6.2.1.2 Screening

Before the interventions begin, a screening assessment must be completed to determine eligibility. Medical records will be reviewed to identify patients who meet the relevant inclusion criteria and do not meet any exclusion criteria. These criteria will be confirmed at the time of enrollment through a questionnaire.

6.2.2 Enrollment, Baseline, and/or Randomization

6.2.2.1 Enrollment

Participants who complete the eligibility screening, consent to participate, and provide an informed consent form for the study will be enrolled. Their contact information will be entered into the database.

6.2.2.2 Baseline assessment

All participants will have a 7-day run-in period during which children are asked to carefully record their OAB-dry symptoms. The purpose of this run-in period is to establish a baseline for evaluating the effect of the intervention measures investigated.

6.2.2.3 Randomization

After completing a comprehensive baseline assessment and meeting the eligibility

criteria, eligible participants will be randomized into one of three different intervention groups. To ensure the highest standard of allocation concealment, the randomization process of this clinical trial will be executed by a designed computer program developed by a project staff member who is not involved in the execution and statistical analysis of this study. The allocation will be securely hidden in sequentially numbered sealed envelopes, which will be entrusted to an inpatient doctor who is not involved in the trial.

6.2.3 Blinding

To minimize assessment bias and ensure the integrity of the study, we will implement rigorous measures to conceal group allocation and hypotheses from outcome assessors and statisticians. We will emphasize the importance of minimizing assessment bias to assessors and regularly review the blinding status to strictly maintain blinding. In addition, the data analyst will only be allowed to review coded data with participant names replaced by numbers.

6.2.4 Follow-up Visits

Patients will undergo regular follow-ups with an outpatient physician. Follow-up assessments will include measurements of urination frequency, urgency score, lower urinary tract symptom score, nocturia frequency, serum vitamin D levels, and other clinical laboratory parameters, such as hematology, biochemistry, and urinalysis.

6.2.5 Completion/Final Evaluation

The effectiveness of various intervention measures for pediatric OAB-dry will be evaluated by comparing changes in the primary urinary frequency and urgency scores between the intervention groups.

7. SAFETY ASSESSMENTS

This study includes clinical interventions, and the primary focus of safety evaluation will be on assessing the adverse effects and exacerbation of clinical symptoms after oral administration of the drug. Patients will be encouraged to report any related complications or adverse events directly to us.

7.1 Specification of Safety Parameters

Participants were required to take solifenacin and high-dose VDS as part of the study. Anticholinergic side effects, including dry mouth, blurred vision, constipation, and nausea, among others, may occur due to solifenacin use. If any of these symptoms occur, the medication will be discontinued, and the participant will be removed from the study. Over-supplementation of vitamin D can lead to symptoms of vitamin D toxicity, including anorexia, nausea, diarrhea, constipation, irritability, and somnolence. Any occurrence of these adverse drug reactions or serum vitamin D levels exceeding 100 ng/mL will be evaluated as unsafe.

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Enrolled participants will undergo follow-up visits every four weeks. These visits

will include a questionnaire and physical examination to assess any drug-related adverse effects, as well as a test of their serum vitamin D levels. In case any adverse effects arise, safety parameters will be promptly evaluated.

7.3 Adverse Events and Serious Adverse Events

The study may result in adverse events related to medication side effects of solifenacin and high-dose VDS. Adverse events and serious adverse events will be classified based on the severity and intensity of the side effects.

7.4 Reporting Procedures

In the event of any adverse event, investigators will be notified within 24 hours.

7.5 Follow-up for Adverse Events

In the event of an adverse event, patients will be advised to seek medical advice immediately.

7.6 Safety Monitoring

As mentioned above.

8. INTERVENTION DISCONTINUATION

If any drug-related adverse effects or worsening of OAB symptoms in children during the intervention period occur, intervention measures should be discontinued. Otherwise, the intervention measures will start eight weeks after enrollment and will

continue until the final data collection. At this point, study participants can withdraw their participation from this study.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

Objective: The aim of this randomized controlled trial is to examine the effectiveness and safety of high-dose VDS plus SU for managing OAB-dry symptoms in pediatric patients with low vitamin D levels (<35ng/mL).

Specific Aim 1: The main outcome is the difference in post-treatment improvement between children who receive high-dose VDS plus SU and those who receive solifenacin plus SU or SU monotherapy for OAB-dry symptoms. The study expects that high-dose VDS plus SU will lead to more improvement than SU monotherapy and solifenacin plus SU. Furthermore, SU monotherapy will result in less improvement than solifenacin plus SU.

Specific Aim 2: The secondary outcome is the safety profile of high-dose VDS plus SU for OAB-dry symptoms in pediatric patients. The study anticipates that the safety profile of high-dose VDS plus SU will be similar to that of solifenacin plus SU or SU monotherapy at follow-up.

The study design is an 8-week randomized controlled trial, and all treatments will be administered in a group setting. Pediatric patients with OAB-dry symptoms will

undergo 8 weekly sessions of high-dose VDS plus SU, solifenacin plus SU, or SU monotherapy. Independent assessors will evaluate the patients before the treatment, at mid-treatment (4-week follow-up), and at the 8-week follow-up to test the expectations. To account for attention, time, and group effects, all SU sessions will be administered in a group setting.

9.2 Sample Size and Randomization

Before we design the current trial, we did a preliminary study with the support of the Program for Youth Innovation in Future Medicine at Chongqing Medical University. This was to check if we could do the trial successfully and recruit enough participants. We recruited children who were between 5 and 18 years old and who had symptoms of urgency and urinated at least 9 times per day as recorded in a 7-day diary. A diary is a tool where participants record their symptoms and behaviors over a period of time. They chose to whether receive either high-dose VDS plus SU therapy for eight weeks, which is our new intervention. The Department of Pediatric Urology at the National Center for Clinical Medical Research (Chongqing) collected bladder diaries from participants before and after the treatment to evaluate how well the treatment worked. The main outcome was the change in how often they urinated, and other outcomes that we measured were how severe their urgency was, how often they woke up at night to urinate, and how bad their symptoms were using the PLUTS score. The PLUTS score is a tool that measures different aspects of lower urinary tract symptoms in children.

We also measured how much their symptoms bothered them and how their health-related QoL was affected by their condition using the QoL score questionnaire. The QoL score is a tool that measures how satisfied participants are with their health and well-being. We also asked participants to rate how much they improved and how satisfied they were with the treatment using validated tools. The results of our preliminary study were positive and showed that our new intervention was promising. This encouraged us to continue with our main trial and test our intervention further. We created and finalized the study protocol, which is the document that describes the details and procedures of our study. We developed all data collection tools and forms, which are the tools and documents that we use to collect and record data from participants. We obtained IRB approvals at the National Clinical Research Center for Child Health and Disease (Chongqing, China), which are approvals from a committee that oversees the ethical aspects of our study and protects the rights and welfare of participants. We offered standard training to all coordinators and interventionists, who are the people who help us run the study and deliver the intervention to participants. Our preliminary data helped us to determine how many participants we needed for our main trial.

The primary outcome in our study will be the change in median voiding frequency from enrollment to 4-week follow-up and 8-week follow-up. In our previous feasibility study, the mean number of voids per day was 16.0 (SD, 5.0) at enrollment. We used the power analysis program PASS 15.0 to estimate the sample size of this randomized controlled trial. A sample size of 87 participants per group was needed to detect a

difference of 1.5 voids per day between groups at the 0.05 level of significance, achieving more than 95% power, assuming an estimated SD of 5.0 after 8 weeks of treatment. We plan to recruit 97 participants per group, anticipating a 10% follow-up attrition, to ensure adequate power for the trial, for a total of about 300 participants. Our sample size estimations are supported by our previous trial.

Independent assessors will be kept blind to the treatment allocation throughout the study. Patients will receive a reminder script emphasizing the importance of not revealing their treatment information before the assessment. Randomization codes will be created by the study statistician before recruiting the first participant in the trial. Data analysis will not start until after the study's completion.

9.3 Definition of Populations

We will use different ways to summarize and analyze our data based on the type of outcome and variable that we are interested in.

9.3.1 Intention-to-treat (ITT) analysis

For outcomes that are continuous variables, which are numerical and can take any value within a range, we will use the ITT approach, which is a way of comparing the groups based on their original random assignment to a treatment, regardless of what treatment they actually received or completed. This helps to avoid bias from factors that may influence the results, such as dropout, which is when subjects leave the study before it ends, or crossover, which is when subjects switch to a different treatment than

they were assigned to. The ITT approach is also simpler than other ways of analyzing the data that need to consider whether the subjects followed the treatment as planned or not.

9.3.2 Per protocol (PP) analysis set

For outcomes that are categorical variables, which are non-numerical and can belong to one of several groups or categories, we will use the PP analysis set, which is a subset of subjects who followed the protocol without major deviations that could affect the outcomes. The protocol is the plan or procedure that we use to conduct the study. Deviations are when subjects do not follow the protocol as expected. For example, this includes patients who used other medications that were not allowed by the protocol or who did not follow the protocol properly. We will decide who to include or exclude in the PP analysis set before we lock the database, which is when we finalize the data after checking and cleaning them.

9.3.3 Safety analysis set

For safety analysis, which is when we assess the safety and side effects of the treatment, we will use the SS set, which is a subset of all patients who took at least one dose of the study drug and have safety records in the database. The database is where we store and manage our data electronically. We will only use measured values for this analysis, which are values that we actually observed and recorded for each subject. We will also do a sensitivity analysis using the PP analysis set, which is when we test how

robust our results are to different assumptions or scenarios.

9.4 Interim Analyses and Stopping Rules

During the study, we will not perform any interim analyses, which are analyses that are done before the end of the study to check the progress and results. We will only do interim analyses if the Institutional Review Board (IRB) requests them. If any SAEs occur during the study, we will report them to the IRB as quickly as we can follow their instructions. SAEs are events that are harmful or life-threatening to the subjects and that are unexpected, serious, and related to the intervention that we are testing. The IRB will have the responsibility to review the study and recommend whether to terminate it or not based on its assessment of the risks and benefits for the subjects. The IRB will do this if it determines that the subjects are exposed to more harm than good at any point during the study.

9.5 Outcomes and data Analyses

We will employ diverse measures to describe the characteristics of our data, depending on their nature. For numerical data that are continuous and can take any value within a range, we will use the mean (the arithmetic average of all values), the SD (a measure of how much the values deviate from the mean), the median (the central value when the data are sorted from the lowest to the highest), and the interquartile ranges (IQRs) (the range containing the middle 50% of data). For non-numerical data that are categorical and can belong to multiple groups or categories, we will use the

frequency (the number of occurrences for each category) and the percentile (the proportion of data that falls below a specific value or category).

We will examine our data for any outliers or missing values that could influence our analysis. Outliers refer to values that differ significantly from the majority of the data and may indicate errors or exceptional cases, while missing values are data points that are unavailable or not recorded. Although we will employ effective data collection and management practices, missing values may still occur due to various factors.

To estimate the missing values for continuous data, we will use multiple imputation. This approach replaces the missing values with plausible estimates based on the other available data. We will assume that the missing values are missing completely at random, meaning that their occurrence does not depend on any variable, including their value. We will also conduct a sensitivity analysis to determine if the missing values affect our results or conclusions.

9.5.1 Primary Outcome

The study aims to determine the primary outcomes of urinary frequency among three groups of participants. The frequency of urination will be recorded at three different points in time, i.e., when the participants enroll in the study, four weeks after starting the treatment, and eight weeks after starting the treatment. We will compare the frequency of urination between the groups at each time point, and assess changes in urination frequency from enrollment to four weeks after starting treatment. To record

urinary frequency, we will use a bladder diary, and these outcomes will be considered continuous variables, with any positive integer value within a specified range.

To analyze the data, we will use the intention-to-treat approach to avoid bias from factors such as dropout or crossover. We will also handle missing data before analysis. Statistical tests will be applied to compare the groups based on data distribution and variance, which affects the reliability of results. For normally distributed data with homogeneous variance, we will use ANOVA, followed by post-hoc comparisons and Bonferroni correction to reduce Type I errors. We will report data changes using mean and SD and differences between groups using point estimation and 95% confidence intervals (95%CI).

For non-normally distributed data with heterogeneous variance, we will use the Kruskal-Wallis test to compare groups. Post-hoc comparisons between groups will be done using the Wilcoxon rank sum test, and p-values will be adjusted by Bonferroni correction. Data changes will be reported using median and IQRs, and differences between groups using the Hodges-Lehmann (HL) estimate of location shift and 95% confidence intervals (95%CI). P-values will also be adjusted for multiple comparisons to avoid false positives, and a two-tailed P-value less than .05 will be considered statistically significant.

9.5.2 Secondary Outcomes

We will measure and compare secondary outcomes that are important for our study,

even though they are not the main outcomes. These outcomes comprise different types of variables, including continuous variables that are numerical and can take any value within a range. Some of the secondary outcomes that we will measure are the average change in urgency severity, the maximum change in urgency severity, the frequency of nocturia, the impact on the participants' QoL, and the severity of LUTS using the PLUTS score. We will measure these outcomes from the time participants enroll in the study to 4 and 8 weeks after they start the treatment. We will compare these outcomes and their changes over time between the groups.

To compare the groups, we will use various statistical tests based on how the data are distributed and how much they vary between the groups. The data's distribution describes how they are shaped and spread out and determines the statistical tests we can use. The variance of the data shows how much the values differ from each other within and between the groups, affecting the reliability of the results.

For normally distributed data with homogeneous variance, we will use the ANOVA test, which compares three or more groups with continuous data to identify significant differences between them. We will report the data and their change using mean and SD, which show the average and variability of the data, respectively. Additionally, we will perform post-hoc comparisons between groups to see which specific groups are different from each other. We will report these comparisons using point estimation and 95% confidence intervals (95%CI), which show how precise our

estimates are and how likely they are to include the true value. An adjusted P value will be reported for each comparison to avoid false positives.

For data that are not normally distributed or have heterogeneous variance, we will use the Kruskal-Wallis test to compare the groups. The Kruskal-Wallis test is suitable for comparing three or more groups with continuous data that do not meet the assumptions of ANOVA. We will report the data and their change using median and IQRs, which show the middle and range of the data. Additionally, we will perform post-hoc comparisons between groups using the Wilcoxon rank-sum test, which can compare two groups with continuous data that do not meet ANOVA assumptions. We will report these comparisons using the HL estimate of location shift and 95% confidence intervals, which show how much one group differs from another group in terms of location or central tendency. We will also report an adjusted P value for each comparison.

We will assess and compare how participants perceive their improvement from enrollment in the study to 4 and 8 weeks after treatment initiation. This subjective outcome reflects how participants feel about their condition and treatment, which we will measure using a rating scale with categories such as much better, better, about the same, or worse. Since this is non-numerical, ordered categorical data, we will use the PP analysis set to analyze the subset of subjects who adhered to the protocol without major deviations. We will apply the Kruskal-Wallis test to compare groups based on their ratings of improvement at different time points, reporting the data using frequency

and percentile. We will use a two-tailed P-value to determine statistical significance, setting a cutoff of less than .05, unless specified otherwise.

Additionally, we will record and compare participant satisfaction with the current treatment and their willingness to receive another therapy. This dichotomous outcome measures whether participants answered yes or no to whether they would like to receive another therapy. We will use the PP analysis set and the chi-square test to compare the groups based on their responses at different time points. We will report the data using frequency and percentile, and consider a P-value of less than .05 to be statistically significant, unless specified otherwise.

9.5.3 Safety analysis

The safety analysis will be performed using the Safety Analysis Set, which will assess treatment-emergent adverse events (TEAEs). Detailed recordings of all severe adverse events and TEAEs leading to discontinuation from the study will be included. Changes in clinical laboratory parameters and vital signs at each scheduled time point from baseline will be summarized according to treatment groups.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

In our study, we intend to measure and compare different outcomes using various tools, such as questionnaires and rating scales. Participants will be required to answer

questions or rate their symptoms or behaviors on a scale. To minimize bias and increase the validity of the results, clinicians and independent evaluators will be trained to administer the questionnaires and scales without knowing which treatment group each participant belongs to. We will take steps to ensure that our data is accurate and that we adhere to the protocol. In cases where children can report their symptoms well, we will consider both what the child and parent say equally, and discrepancies between the two reports will be investigated further.

We will utilize QR codes to enable participants to fill out self-report forms, which can be scanned by a smartphone or tablet and linked to a website. Program alerts will be used to remind participants to answer all questions and enter valid values. We will maintain confidentiality and compliance throughout the study, labeling data by subject number, visit number, and date of visit only in our database. Participants will be given a unique ID number that only we can link to their names. To ensure data security, we will encrypt and password protect all electronic data transfers. Hard copies of personal health information and consent forms, as well as study measures on paper, will be kept in locked drawers by another researcher.

The research team will conduct regular reviews to evaluate the quality and completeness of data collected during the study, including patient intake and retention progress, patient compliance with visits, evaluations, and treatments as per the protocol, any adverse events or safety concerns, and the accuracy and completeness of key data

elements required to characterize patients. These reports will assist the research group in determining the effectiveness of data capture and processing methods to facilitate scientifically sound analyses.

10.2 Data Management

The biostatisticians will manage the study database, while the principal investigators (Xing Liu) will oversee the researchers and data management staff to ensure the quality and execution of the study. X Wang, MD, will supervise the data management and quality. The collected data will include various assessments, such as screening data, clinician- and patient-rated assessments, fidelity, visit adherence, and safety data, as well as interrater reliability and certification data. Weekly reports will track enrollment, completion, attrition, subject progress, and critical assessments. Baseline characteristics, protocol adherence, and other issues will be monitored using additional reports as necessary.

Data will be collected directly by patients and study staff through a QR code, which links to a website. The data will be exported to a relational database in Microsoft software for systematic querying and checking. Paper-based data will be entered by 2 independent staff members to prevent errors or missing data and verified by another staff member at the end of the study.

Monthly reports will monitor enrollment, completion, attrition, subject progress, and critical assessments. Additional reports will monitor baseline characteristics,

protocol adherence, and other issues. Data will be de-identified using subject number, visit number, and date of visit to comply with local privacy requirements. Electronic data will be encrypted and password-protected, with only ID numbers being used to identify subjects. De-identified data will be transmitted for data analysis. Consent forms and hard copy protected health information will be kept in locked cabinets in a secure office.

10.3 Quality Assurance

10.3.1 Training

The study will provide training to participants on how to use bladder diaries and rating scales. The individuals providing the training, called SU trainers, will be unaware of the treatment assignment. The diagnostic and efficacy evaluations will be reliable and valid, ensuring that the results of the study are trustworthy. To ensure that all raters, including children and parents, are providing accurate ratings, they will receive specific training on the rating scales. The training will focus on issues such as overestimation, underestimation, and recall period. The SU trainers will also administer SU sessions. The reliability and integrity of these sessions will be established and maintained through specific training, certification, and weekly meetings. All SU trainers will be experienced clinicians who have met the same criteria for teaching behavioral therapy. Any new SU trainers will undergo training and have their sessions reviewed by an SU expert for agreement. Weekly meetings will also help reduce the potential for training

bias. By implementing these procedures, the study aims to ensure that participants understand and effectively use behavioral therapy.

10.3.2 Protocol Deviations

Any protocol deviations will be recorded in the protocol deviation log and reviewed with the study PI at weekly meetings.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 IRB Review

This protocol and the informed consent document have been reviewed and approved by the institutional review board of Children's Hospital of Chongqing Medical University. This Clinical Trial will be conducted in compliance with national laws and regulations, as well as any applicable guidelines.

11.2 Informed Consent Forms

In accordance with local regulations, participants in the study will be required to provide informed consent. For children over the age of eight, both the child and legal guardian must sign the informed consent form, whereas for children under eight years of age, only the legal guardian needs to sign.

To ensure that each participant has a full understanding of the study's nature, potential risks and benefits, and purpose, the study team will provide both oral and written information. Participants will be informed that they have the right to withdraw

from the study at any time and will be given sufficient time to consider the information provided and ask questions before providing their signed and dated informed consent. The original, signed informed consent forms will be stored in the Investigator's Study File, and a copy will be given to the subject. The informed consent form will also describe any incentives for study participation and any provisions for subjects who may experience harm as a result of their participation.

Ensuring informed consent is critical to upholding ethical standards in research, and these procedures will help ensure that each participant fully understands the study and has given informed consent before participating.

11.3 Participant Confidentiality

To protect the confidentiality of participants, any data, forms, reports, or other records that are taken away from the research site will be identified only by a participant identification number (PID). These records will be stored in a secure and locked file cabinet, and computer programs will also use only PIDs for data entry and networking. The information will not be disclosed without written permission from the participant, except for monitoring by the IRB.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB or other government agencies as part of their duties to ensure that research participants are protected.

11.5 Monitor

The monitoring plan should be prepared by the monitor before the start of the study, and monitoring should be performed in accordance with this plan during the study. The IRB is responsible for monitoring the proper execution of the study and ensuring the consistency and availability of data through a management and observation plan. The primary role of the monitoring team is to assist investigators in maintaining high quality standards in ethics, scientific methods, techniques, and laws. Additionally, the monitoring team must ensure that the study is conducted in compliance with the Clinical Trial Protocol, Chinese GCP, and SOP. The monitoring team will inspect the following during the study's monitoring: subject recruitment and follow-up, SAE records and reports, the distribution of investigational drugs, protocol compliance for, and data quality.

12 AUDITS

The IRB auditor will conduct a comprehensive audit of all clinical study-related activities and documents to evaluate compliance with the protocol, standard operating procedures, and relevant regulations. The accuracy, completeness, and timeliness of the recorded data will also be evaluated. To ensure impartiality, the audits will be conducted by personnel or group who are not involved in the clinical study.

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15. SUPPLEMENTS

Pediatric Lower Urinary Tract Symptom Score (PLUTS score)

ID: _____

1.Does your child wet during the day?	□ NO	□Sometimes	□1-2times/day	□ Always
	0	1	3	5
2. How wet is your child during the day?	□Damp underwear	□ Damp pants only	□ Pants soaking wet	
	1	3	5	
3. Does your child wet during the night?	□ No	□ 1–2 Nights/ week	□ 3–5 Nights/ week	□6–7 Nights/ week
	0	1	3	5
4. How wet is your child during the night?	□ Damp bed sheet only		□ Bed sheets soaking wet	
	1		4	
5. How many times does your child void?	□ Less than7/day		□ 7 or more than 7/day	
	0		1	
6. My child strains during voiding.	□ No		□ Yes	
	0		3	
7. My child feels pain during voiding	□ No		□ Yes	
	0		1	
8. My child voids intermittently	□ No		□ Yes	
	0		2	
9. My child needs to go back voiding soon after finishes his/her pee.	□ No		□ Yes	
	0		2	
10. My child has a sudden feeling of having to urinate immediately	□ No		□ Yes	
	0		1	
11. My child holds by crossing his/her legs.	□ No		□ Yes	
	0		2	
12. My child wets on the way to the toilet	□ No		□ Yes	
	0		2	
13. My child misses his/her bowel movement every day.	□ No		□ Yes	
	2		0	
14. If your child experiences symptoms mentioned above, does it affect his/ her family, social or school life?	□ Not at all	□ Sometimes	□ Seriously affects	
	0	1	5	

Total score: _____ Date: