Principles and Practice of Clinical Research
A Global Journal in Clinical Research

PPCR
ISSN: 2378-1890

Vitamin D Supplementation for the Prevention of Recurrent Urinary Tract Infection in Women - PROUD: A Randomized Controlled Trial

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Principle and Practice of Clinical Research: Group 3. Harvard Medical School. 2015.
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Received December 3, 2015; accepted January 21, 2016; published March 15, 2016.

Abstract:
Urinary tract infection (UTI) due to Escherichia Coli is considered the most commonly acquired bacterial infection in women. Currently used treatments for UTI prevention have conflicting evidence regarding efficacy. There is minimum information regarding the use of Vitamin D and its effect on modulating the innate and adaptive immune system, favoring the production of endogenous antimicrobial peptides such as Cathelicidin. It has been suggested that inducing Cathelicidin in the bladder is a potential complement in preventing UTI. This clinical trial aims at testing the use of Vitamin D in women diagnosed with recurrent UTI due to Escherichia Coli for its prevention. This will be a randomized, double blind controlled trial, with a multicenter approach.

Keywords: *Urinary Tract Infection (UTI); Women; Prophylactic Antibiotic; Vitamin D; Placebo.*

DOI: http://dx.doi.org/10.21801/ppcrj.2016.21.4

INTRODUCTION

Urinary tract infection (UTI) is considered the most commonly acquired bacterial infection both in outpatient and inpatient settings (Moue, Aktaruzzaman, & Ferdous, 2015). Causative pathogen may vary but Escherichia coli is still the most common causative pathogen in community- acquired uncomplicated UTI cases (Moue et al., 2015). It has been said that 11% of adult women in the United States will have an UTI each year (Betsy Foxman & Brown, 2003), with peak incidence varying from 20 to 24 years of age, which represents 18.6% of total cases observed. Furthermore, around 5% (Cunningham & Lucas, 1994) of the women who present with an initial UTI will have recurrent UTI (rUTI), defined as three or more uncomplicated UTIs in a 12-month period, which can be due to either a "relapse" after ceasing treatment or to a "reinfection" with a different organism, also after ceasing the initial treatment ("Adult UTI," 2015). Although considered to be a benign condition, rUTI can have a significant impact on quality of life.

Acute, uncomplicated UTIs are generally qualified as benign conditions. However, severe infections may require hospitalization, and untreated UTI can lead to permanent kidney damage from an acute or chronic infection (pyelonephritis), with increased risk of women delivering low birth-weight or premature infants. Also, the high prevalence of UTIs, combined with the costs associated with medical intervention, has significant financial ramifications (Betsy Foxman, 2002), which highlights the importance of preventing UTI recurrence.

The current guidelines suggest the use of continuous low-dose antibiotic prophylaxis as an effective way of preventing UTIs ("Adult UTI," 2015, "Brazilian guidelines for rUTI prophylaxis," 2011). However, there are many drawbacks of prolonged antibiotic prophylaxis, such as inducing resistance to the antibiotic, changes on the normal microorganisms found in the human body and

adverse effects (vaginal and oral candidiasis, skin rash and nausea), leading to frequent withdrawal and decreased adherence (Metz, McGuinness, & Harris, 1998) (Dason, Dason, & Kapoor, 2011). In 2004, a Cochrane Review showed that patients with frequent UTIs who take prophylactic antimicrobial agents for extended periods (more that 6 months) decrease their episodes of infections during prophylaxis, but the rate of infection returns to pretreatment rates when prophylaxis is stopped ("Brazilian guidelines for rUTI prophylaxis," 2011, "Recurrent urinary tract infection in women," 2015) with the presence of side effects due to the treatment.

As an alternative to antibiotic use, cranberry has also been studied, however without success, as indicated in the 2012 Cochrane systematic review (Jepson, Williams, & Craig, 2012). The results showed are inconclusive about the protection effect provided by cranberry therapy used for UTI profilaxy. Thus, other preventive strategies that can decrease the need for antibiotic therapy are required. Concerns about Vitamin D deficiency emerged when some studies observed the association of lower levels of Vitamin D with autoimmune diseases and infections. Hertting et al (B. Foxman et al., 2000) showed that Vitamin D could induce cathelicidin in the bladder, which is a human antimicrobial peptide that is expressed and secreted by bladder epithelial cells, protecting the urinary tract from infection. They observed that Vitamin D per se did not up- regulate cathelicidin in serum or in bladder tissue of the women, but when the bladder biopsies were infected with uropathogenic E. coli (UPEC); a significant increase in cathelicidin expression was observed after 25D3 supplementation. These findings suggest that 25D3 might be a likely complement in the prevention of UTIs; however, there is still a lack of evidence that the correction of serum levels of 25 (OH) Vitamin D could prevent the recurrence of UTIs. (Anty et al., 2014; Dason et al., 2011; Jernberg, Löfmark, Edlund, & Jansson, 2010; Quraishi et al., 2015)

Therefore, due to the importance and prevalence of rUTI, the lack of prevention strategies with no important drawbacks and the lack of knowledge about Vitamin D role in UTI, we will conduct a 2-arm parallel, double blind RCT in order to determine the protective role of Vitamin D in recurrent UTIs in women older than 18 years old during a one-year period.

Mechanisms

Urinary pathogens have characteristics that aid to their survival, pathogenicity and virulence; these include fimbriae, pilli, flagella and siderophores to scavenge iron. The innate immune system counteracts them through antimicrobial peptides (AMPs), cytokines and phagocytes. AMPs directly lyse the microbes' membranes, and also function as chemoattractants for leucocytes. AMPs include cathelicidin and α and β defensins. Cathelicidin is a linear peptide expressed in all epithelial surfaces and it is induced by local lesion or infection (Zasloff, 2007). As other examples of anti-infective proteins, the Tamm-Horsfall protein inhibits E. coli fimbriae from adhering to urothelium. Lactoferrin and lipocalin restrict iron availability (Zasloff, 2007).

Cathelicidin is expressed within minutes following infections and it is continuously synthesized afterwards. Cathelicidin-absent mice present an increased number of bacteria in bladder epithelium and ascending infections (Morrison, Kilanowski, Davidson, & Dorin, 2002).

The stimulation of Toll-like receptor (TLR) by microbes induces the expression of Vitamin D receptor (VDR) and 1,25-dihydroxyVitamin D-1 α hydroxylase (CYP27B1). CYP27B1 converts 25Vitamin D (the precursor of Vitamin D) to 1,25 Vitamin D (active form). VDR bound with Vitamin D enhances the expression of AMPs like β defensin and cathelicidin (Borella, Nesher, Israeli, & Shoenfeld, 2014). Vitamin D's pleiotropic treatment effects have been observed in several diseases including, tuberculosis, diarrhea caused by Shigella (Raqib et al., 2006), asthma and Hepatitis C infection (Kitson & Roberts, 2012).

Vitamin D adequacy is controversial with varying levels depending on assay and laboratory. However, there is general consensus that its deficiency is related to several diseases. For example, aberrant regulation of innate immunity to enteric bacteria has been associated with Vitamin D deficiency and inflammatory bowel disease. Vitamin D deficiency in chronic renal disease has been associated to macrophage infiltration and inflammatory damage as well as predisposition to bacterial peritonitis.

Regarding urinary tract infections, earlier studies have reported a statistically significant inverse correlation between low serum Vitamin D levels and urinary tract infections in premenopausal women, as well as in healthy children. Low serum Vitamin D levels are significantly associated with a higher prevalence of ascending infection (pyelonephritis) (Nseir, Taha, Nemarny, & Mograbi, 2013). Furthermore, a study in postmenopausal women demonstrated Vitamin D supplementation increased cathelicidin expression ex vivo in bladder biopsies with E. coli infection (Hertting et al., 2010) (Ralph, Ralph, Lucas, & Norval, 2013; Tekin et al., 2015).

Need for a trial (innovation aspects)

UTI is a very prevalent disease especially in women, with an estimated of 150 million urinary tract infections per annum worldwide and cost the global economy in excess of 6 Billion US dollars (Moue et al., 2015). With an incidence of UTI of 12.1%, there is a 5% rate of recurrences usually managed by low and continuous dose of antibiotics. Medical literature has found a relationship between Vitamin D and a stimulation of cathelicidin in bladder mucosa, which might decrease the risk of recurrences in UTI. Earlier studies have reported a statistically inverse correlation between low serum Vitamin D levels and UTI, as well as a higher prevalence of ascending infection. This trial proposes to assess the relationship between Vitamin D supplementation and the reduction of recurrant E.Coli UTI in women since it has never been studied before. The findings might establish a new intervention to prevent rUTI, as well as to decrease costs and the burden in quality of life caused by rUTI among the adult women population.

Significance/impact of study

This study might add critical information in the management of UTI in adult women since this is a very prevalent disease that poses important aspects in quality of life, as well as costs involved in the treatment. Serum supplementation of Vitamin D is a safe strategy used for other conditions, and might play an important role in UTI.

METHODS

Primary question

"Is Vitamin D supplementation 2,000 IU/day more effective than placebo for the prevention of E. Coli rUTI in 18 year-old women or older with past confirmed urinary tract infection on more than 2 occasions?"

P: Women 18 and older with 2 or more positive urine cultures due to E. Coli

I: Vitamin D supplementation 2,000 IU/day

C: Placebo

O: Recurrent symptomatic urinary tract infection (UTI) secondary to E. Coli (symptoms of cystitis, fever, pyelonephritis, leukocytosis PLUS a positive urine culture with E. Coli)

T: 1 year

Study objectives

The Primary Objective is to determine if Vitamin D supplementation of 2000 UI reduces recurrence of E. Coli Urinary Tract Infection (UTI) in women of 18 years old and older, with past-confirmed 2 or more episodes of E. Coli UTI.

The Secondary Objectives are: 1) Incidence and possible effect of Vitamin D supplementation on the prevention of ascending infection (pyelonephritis, perinephric abscess in this population; 2) Incidence and possible effect of Vit D supplementation for the prevention of new UTI due to another microorganism (non-E. Coli); and 3) Number of recurrent UTIs in studied population during the established period of time.

Trial design

This will be a randomized, double blind controlled trial (rUTI defined as 2 or more urinary tract infections in past 6 months or 3 or more urinary tract infections in the past 1- year with culture positively for E. Coli only). Target population are women of age 18 and older who have been diagnosed with rUTI due to Escherichia coli from primary care, urology, infectious diseases and gynecology clinic to participate in this trial.

This will be a 2-arm parallel design and patients will be randomly allocated in 1:1 to either receive placebo or Vitamin D at a dose of 2000 IU/day and will be followed up for 1 year for recurrence of urinary tract infection due to E. Coli.

Patients will have a follow up with visit scheduled every 4 months after the initial visit. Patients will have a complete history and physical examination including any episodes of urinary tract infections and complete laboratorial data, collected at every visit and will include plasma ionized calcium, phosphate, blood urea nitrogen, creatinine, urinary calcium levels, liver function tests (transaminases, alkaline phosphatase, total bilirubin, serum protein and albumin) and serum 25 (OH) Vitamin D will be measured at every visit.

Subjects will be educated to visit their respective physician if they present any symptom suggestive of a urinary tract infection (urgency, dysuria, lumbar pain, fever, vomits). If symptoms develop in non-office hours, instructions of visiting the emergency room of the respective study center are given. Emergency physicians will be instructed to report the event to the physicians involved in the trial, and to take a sample for urine analysis and culture. Also, patients are instructed to record and inform to the physician office the event via telephone or e-mail.

Study setting

We will be inviting patients from primary care, infectious disease, urology, gynecology clinics for this study. This will be a multicenter trial involving 5 sites in Brazil: Sao Paulo, Salvador, Rio de Janeiro, Porto Alegre, and Brasilia.

Eligibility criteria

The criteria for inclusion of patients are the following: women aged 18 and older, recurrent UTI: 2 uncomplicated UTIs in 6 months or 3 positive cultures within the preceding 12 months, and outpatient management.

On the other hand, the criteria for exclusion of patients are: low-dose antibiotic prophylactic therapy or cranberry juice consumption / supplementation; urinary incontinence, urinary tract obstructions, malformations, malfunctioning or instrumentation (any cause) (these clinical situations increase the risk for UTI); pregnancy, diabetes Mellitus, immunodeficiency (HIV, Systemic corticosteroid therapy, immunomodulation drugs, transplant patients), use of topic estrogens; liver failure (Thiem, Olbramski, & Borchhardt, 2013) and renal function impairment (Kim & Kim, 2014).

Information regarding exclusion criteria will be obtained from past medical records and diagnostic studies performed previously. If there is any suspicion for a given exclusion criteria but there is no evidence from the past to support it, the patient must be evaluated by primary physian with the given concern. These criteria were highly discussed and evaluated in literature. Some are taken into account because they can present immunological alterations, increase UTI risk propensity, increase risk for adverse effect of Vitamin D supplementation or dosage adjustment needed for certain poplation. Consequently, exclusion criteria if not taken into account will lead to confounding and unappropiate results.

Recruitment Strategy

The recruitment strategy will include: 1) Public awareness campaigns will be launched, and support from local medical societies will be requested. By using these approaches, more eligible patients will be recruited, physician referral will increase and recruitment costs will be reduced. 2) Health facilities: E-mails and/or invitation letters will be sent to patients on their data bases, especially those on Urology, Nephrology and Ob/Gyn sections. 3) Trial information will be advertised in women's magazines and journals.

Our plan to approach patients includes the following action items: 1) Delivering of trial information: using a video or other audiovisual material that includes trial purpose, benefits, risks, randomization and value to society, also print out brochure. 2) Consent form: describing Vitamin D benefits for the prevention of UTI, emphasizing that episodes of UTI may decrease. Neutrally framed information about side effects will be provided and financial disclosure will be included. 3) Previously trained and easily accessible recruiters will work in patients' approach.

Interventions

Eligible patients will be randomized as indicated previously. Vitamin D3 (cholecalciferol) will be purchased from Kirkland Pharmaceuticals. Placebo tablets will be manufactured by Catalent pharma Solutions. Both Vitamin D3 and placebo tablets will be delivered to a centralized pharmacy and will be distributed from there to the sites to maintain blinding.

Study subjects will receive their allocated treatment in form of capsule package on the initial visit after screening and collection of necessary samples has been done. They will be instructed to start their allocated treatment on the same day and take their capsules once a day at the same time of the day. All personnel will be blinded to the contents of capsule package.

Overdose and intoxication

Taking into account the possibility of an overdose of Vitamin D Supplementation, at every scheduled visit, the study center will check the adherence and if the study participant correctly followed the prescription. The estimated toxic dose of Vitamin D should be greater than 100,000 IU per day for, at least, one month (Araki et al., 2011). In case of suspicious overdose by clinical or laboratory data, this should be reported as an adverse event and the study participation should temporary suspend trial medication. A complete laboratorial data will be collected by the study center: plasma calcium ionized, phosphate, creatinine, 25 (OH) Vitamin D levels and urinary calcium excretion. If the study center doctor understand as pertinent, can be also included: 1.25-dihydroxyVitamin D; 24.25-dihydroxyVitamin D, parathyroid hormone (PTH); serum collagen type 1 cross-linked C-telopeptide (CTX) (van den Ouweland, Fleuren, Drabbe, & Vollaard, 2014). In that case, an additional medical appointment in the study center should be scheduled within a maximum of 5 days.

The potential intoxication level of 25 (OH) Vitamin D in serum is over 250 nmol/L indicate potential intoxication (Nasri et al., 2014).This should be reported as an adverse event and the study participant should temporary suspend trial medication.

Hypercalcemia

Hypercalcemia is one of the most serious adverse effects and have high sensibility for Vitamin D intoxication (Jacobsen, Hronek, Schmidt, & Schilling, 2011); as a laboratory value is defined as a serum total calcium levels

greater than 10.3 mg/dL (corrected for albumin). If signs and/or symptoms that can be related to hypercalcemia are identified, including, headache, nausea, vomiting, lethargy, confusion, sluggishness, abdominal pain, bone pain, polyuria, polydipsia, weakness, cardiac arrhythmias (e.g., QT shortening, sinus tachycardia), soft tissue calcification, calciuria, and nephrocalcinosis, this should be reported as an adverse event and the study participant should temporarily suspend trial medication. In that case, an additional medical appointment in the study center should be scheduled within a maximum of 5 days.

Hypercalciuria

It is considered when Urinary calcium excretion greater than 250 mg/day (6.24 mmol/day) in women (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). Increased urinary calcium excretion is the most sensitive indicator for potential Vitamin D intoxication (Jacobsen et al., 2011). This should be reported as an adverse event and the study participation should temporarily suspend trial medication. In that case, an additional medical appointment in the study center should be scheduled within a maximum of 5 days.

Acute liver failure

Defined by King's Colleges Criteria (Holick et al., 2011). Since supplementary Vitamin D2/D3 cannot metabolically be converted in its final active metabolic calcitriol, the study participant should be excluded from the trial. In that case, an additional medical appointment in the study center should be scheduled within a maximum of 5 days.

Renal function impairment

Renal function impairment is defined as an increase of 2 or more times baseline creatinine levels or Clearence Creatinine < 30 by CKD-EPI creatinine equation (O'Grady, Alexander, Hayllar, & Williams, 1989). Since supplementary Vitamin D2/D3 cannot metabolically be converted in its active metabolic calcitriol, the study participant should be excluded from the trial, and extra medical consultation in the study center should be scheduled within a maximum of 5 days.

Allergic Reactions

Look for any signs of an allergic reaction, like rashes; hives; itching; red, swollen, blistered, or peeling skin with or without fever; wheezing; tightness in the chest or throat; trouble breathing or talking; unusual hoarseness; or swelling of the mouth, face, lips, tongue, or throat. These should be reported as an adverse event and the study participant should temporarily suspend trial medication, and extra medical consultation in the study center should be scheduled within a maximum of 5 days (Levey et al., 2009).

Meetings

The initial meeting will include an explanation of the study, including: objectives; advantages of the therapy to help improving the patients' conditions; the study capsule may be Vitamin D supplementation 2,000 IU/daily or placebo; and study blinding. There will be a clear explanation about the low risks and benefits to get emotional commitment from patients. Instructions about: dose timing; storage; whole capsule ingestion; procedure in case of a missing dose. In order to improve adherence, the importance of following the prescription will be emphasized. Suggestion of reminder strategies will be: taking capsules at a time of a specific day of the week; use a call reminder and a weekly pillbox. Patients will be encouraged to discuss the treatment with the family or community. Hand-out to patients will be: one month capsule package; questionnaire about adherence follow-up to be filled during the month; leaflet (FAQ about the study and adherence). Regular calls to the clinic will be helpful in case of problems possibly related to the study such as adverse effects, lost capsules, or for any other query. Information for the follow-up monthly meetings: return the original capsule package with the unused capsules (if any) and the filled questionnaire; notification that there will be a capsule count; some days before the clinic will contact the patient to remember the next meeting and to confirm his/her presence.

The follow-up meetings will occur every 4 months over a 12-month period. They will include a brief discussion about the results and, if necessary, reinforcement of simple strategies for enhancing adherence. Participants will be asked to expose the problems they faced and the items of the initial meeting will be reviewed based on their particular demands. Hand-outs to patients: capsules package and the questionnaire for the next month.

Adherence

The adherence will be assessed through capsule count, self-report questionnaires and blood exams for measurement of Vitamin D serum level at baseline and 4, 8, and 12 months. These adherence data can help the analysis and the interpretation of the trial.

The challenges in adherence are: Need to determine baseline Vitamin D serum level and to determine if checking Vitamin D levels is necessary during the trial. This will ensure therapeutic levels as well as a secondary

check to ensure adherence to medication. Pill tracking may be a problem. And adherence and data analysis: We should plan to do ITT analysis as much as possible and anticipate potential numbers of lost-to-follow-up patients.

The incentives provided to help improve adherence will be: parking stamps, food or grocery stamps; enrollment in quality of life meetings or maybe yoga sessions; pill reminder watch; payment for the antibiotics for the first treatment of the trial; and treatment and physician care for possible recurrent infection.

Strategies to improve adherence

The established Vitamin D3 posology is 2,000UI/day and 14,000UI/week. Between these two options, we choose daily because of the better compliance, persistence and efficacy based on others examples in literature (Chel, Wijnhoven, Smit, Ooms, & Lips, 2008).

The capsules both of intervention and placebo should match imprint, size, shape and color. Also, the smell and taste should be neutral and palatable.

The intervention and placebo have a minimum side effects profiles and the participant should have the support of the research team for adverse effects. The adherence will be assesed through capsule count, self-report questionnaires and blood exams for measurement of Vitamin D serum level at baseline and 4, 8, and 12 months. These adherence data can help the analysis and the interpretation of the trial.

Challenges in adherence

- Need to determine baseline Vitamin D serum level and to determine if checking Vitamin D levels is necessary during the trial. This will ensure therapeutic levels as well as a secondary check to ensure adherence to medication.
- Pill tracking may be a problem.
- Adherence and data analysis: We should plan to do ITT analysis as much as possible and anticipate potential numbers of lost-to-follow-up patients.

Incentives to help improve adherence

- Parking stamps, food or grocery stamps.
- Enrollment in quality of life meetings or maybe yoga sessions.
- Pill reminder watch.
- Payment for the antibiotics for the first treatment of the trial.
- Treatment and physician care for possible recurrent infection.

Outcomes: Primary

Recurrence reduction of E. Coli UTI

Outcomes: Secondary

All outcomes will be assessed in each follow-up visits, which will happen every 4 months (3 visits during 12 months - total duration of trial). Patients will be previously informed and requested to bring any medical record regarding new episode of symptomatic UTI, rUTI and ascending infections, which will be evaluated by the history of hospitalization due to complicated UTI and urinalysis/urine culture reports will be required in order to confirm some of these outcomes.

- Incidence of ascending infection (pyelonephritis, perinephric abscess) (medical records).

The incidence of ascending infection will be assessed, with the analysis metric being the final value. This is a binary outcome, for which assessment will evaluate a yes/no answer for hospitalization due to complicated UTI. Statistical analysis will include comparison of proportions.

UTIs, when properly treated, are generally limited. However, a small proportion of cases, especially when untreated, might evolve to permanent kidney damage from an acute or chronic kidney infection (pyelonephritis), renal abscess, renal impairment, and septic shock. These complications increase the economic burden of these infections and may represent chronic consequences for the patients. Therefore, it is of vital importance to prevent UTI before these impairments occur. The aim is to investigate whether, among the patients that have recurrent UTI, Vitamin D will be able to reduce the incidence of ascending infection, therefore also having interference in the prognostic development of this pathology (Neumann & Moore, 2014).

- New UTI due to another microorganism (non-E. Coli).

The incidence of a new UTI due to another microorganism other than E. Coli will be assessed in order to investigate if Vitamin D has interference in the pathogenesis of other agents. This is a binary outcome, for which assessment will evaluate a yes/no answer with further specification regarding the type of etiological agent.

Although E. Coli is the main pathogen associated with UTI in women, numerous other agents may also lead to impairment of the urothelium, including Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus. Non-E. Coli infection can lead to serious complications, such as kidney scarring, hypertension and renal impairment (Flores-Mireles, Walker, Caparon, & Hultgren, 2015).

- Number of recurrent UTIs.

The number of recurrent UTIs will be assessed, with the analysis metric being the final value. This is a discrete

outcome and the method of aggregation proposed is mean with standard deviation.

There is poor evidence regarding Vitamin D for the prevention and the decrease in episodes of recurrent UTI. From those patients who develop recurrent UTI (25%), the number of recurrences experienced varies from 0.3-7.6 episodes per year (1). It has also been evidenced that most recurrent infections are due to uropathogenic E.Coli (UPEC) (Nosseir, Lind, & Winkler, 2012). There are some studies that have evidenced positive changes regarding urothelial cells when Vitamin D is given and patient remains with normal serum levels thereof. When bladder cells are exposed to Vitamin D, they increase antibacterial effect against UPEC and increase cathelicidin which is a human antimicrobial peptide expressed and secreted be these cells for the protection against infections (Hertting et al., 2010). Consequently, based on physiopathology, Vitamin D may not only reduce microorganism invasion but also the number of episodes per year in these patients.

Hypothesis

Vitamin D supplementation 2,000 IU is better than Placebo reducing E. Coli UTI recurrence in 18 years old and older women with confirmed 2 or more E. Coli UTI episodes.

Timeline

A summary of scheduled assessments for the study is presented in the flowchart. The study data will be collected at the time of each visit and by medical record review.

During the screening visit at any time of day or night, eligible patients with 2 or more positive urine cultures due to E. Coli may be asked to participate.

Eligible patients will receive study medication after meeting inclusion and exclusion criteria. All patients who receive study medication will be followed up for 1 year.

A patient visit may be rescheduled as long as it is within the acceptable time windows of the protocol. Every effort should be made to have the patient adhere to the visit schedule. Patients who prematurely withdrawn form study medication must undergo end-of-study (Visit 5) procedures.

Randomization

Participants will be randomized using the randomization module within REDcap. The participants will be randomly allocated in a ratio 1:1 to either control or treatment group and stratified in 3 groups. Stratification will be done by age: 18 to 30, 31 to 50 and 50 and beyond.

Allocation concealment will be ensured, as the service will not reveal the randomization code until the

Figure 1. Study visits.

patient has been entered into database. Patients' characteristics will have to be entered first and then randomization code will be issued and locked in the database. Another safeguard to ensure allocation concealment will be to separate recruitment and ensure therapy groups randomization.

A patient who gave consent for participation and who fulfills the inclusion / exclusion criteria will be enrolled by the Recruitment Group (RG). In sequence, RG will request the randomization of the patient from the Coordination of Clinical Trials (CCT).

After the CCT generates the allocation sequence using the aforementioned randomization software, strata will be created prior to beginning randomization, using age as a criteria. The CCT will create a randomization list and provide envelopes following the list. Inside each envelope the corresponding code for the Therapy Group (TG) will be enclosed. These envelopes are sealed and opaque and will be stored in a safe place in each center, under the supervision of the CCT.

To implement the allocation concealment the method of envelope is adopted, because of its simplicity, but with some additional procedures to provide greater assurance in the process, such as: the form containing the code of the treatment are involved with aluminum foil inside the envelope to make it impermeable to intense light; the envelope should be opened only after the appropriated data (data of the patient, for instance) are written on the envelope; a carbon paper inside the envelope will transfer the information to the assignment card, thus creating an audit trail (Schulz & Grimes, 2002).

Blinding

This is a double-dummy, double-blinded, placebo-controlled, phase III study. All investigators and subjects will be blinded and will not have access of the assignments defined at enrollment to the treatment arms of the trial. The Core Coordination Center will be the only unblinded group (involving a medical doctor) in this trial responsible for randomization, data management and reviewing laboratory results (notification will be sent if a participant presents with an abnormal result).

FDA guidelines for Urinary Tract Infections (2015) are used for blinding ("Complicated Urinary Tract Infections: Developing Drugs for Treatment Guidance for Industry. Clinical / Antimicrobial.," n.d.), which states that all trials with this pathology should be multicenter and double- blinded unless there is a compelling reason for single-blind or open-label trials. If trials are single-blind or open-label, sponsors should discuss potential biases with the FDA and how these biases will be addressed. Therefore, considering our trial, there is no compelling reason by which we should not perform a double-blind trial.

This study does not foresee emergency unblinding due to the intervention type proposed in it, which is a supplementation of Vitamin D for the prevention of recurrent UTI secondary to E. Coli. No event is expected to require breaking the code for subjects in treatment.

Sample size calculation

The primary outcome for this trial is to determine the effectiveness of Vitamin D 2,000 IU per day supplementation for preventing the recurrence of UTI secondary to E. Coli in 18-year-old women or older that have suffered confirmed urinary tract infections in more than 2 occasions when compared to placebo.

The estimated sample size for this trial is 720 patients. This sample size was calculated with STATA and the following assumptions were made: first, a non-parametric design, since the outcome is categorical/binary (recurrence UTI); second, a 30% UTI recurrence for the placebo group based on the existing literature (Ikäheimo et al., 1996; McMurdo, Bissett, Price, Phillips, & Crombie, 2005); third, that a reduction of recurrence (effect size) of 10% caused by Vitamin D would be clinically relevant; by that, we refer to a recurrence frequency of 20% in the active group; and fourth, we anticipated an attrition rate of 15%, that was used to adjust the estimation.

Determination was based on a two-sided significance level of 5% and 80% power, equally allocated into two arms, with each arm having 360 patients. Assumptions when calculating simple size: alpha = 0.0500 (two-sided), power = 0.8000, p1 = 0.3000, p2 = 0.2000, n2/n1 = 1.00.

Statistical analysis

The intervention arm (Vitamin D supplementation 2,000 IU/day) will be compared against the control (placebo) for all primary analyses. As indicated in the article from Fisher et al (1990), all the patients that participate in the intention-to-treat analyses (ITT) are assigned randomly to their respective groups. For their assignment, factors such as their adherence with the criteria for entry, the treatment provided to them, and eventual withdrawal or deviations from the protocol are not taken into account.

In our case, the analyses will try to assess the effect that Vitamin D supplementation has on the recurrence of symptomatic UTI secondary to E. Coli. The primary outcome is recurrence/non-recurrence of the UTI, and therefore it is binary. The secondary outcomes are: incidence/non-incidence of ascending UTI (binary outcome), incidence/non-incidence of a new UTI due to

another microorganism (binary outcome) and number of recurrent UTIs (continuous discrete outcome).

We will use t-test for continuous outcomes, Chi-squared test for binary outcomes and Wilcoxon Sum Rank Test for discrete outcomes. To assess the effect of Vitamin D supplementation on whether or not the patients will present recurrence of UTI, we will calculate the risk ratios (RRs) with 95% confidence intervals (CIs). In addition to ITT analyses, we will perform a second stratified analysis by age with the purpose of addressing the hypothesis of whether Vitamin D supplementation has a greater effect in older people, whose immune systems are likely to be less effective, this will be done using ANOVA or Kruskal wallis depending of the normality of the groups. For all tests we will use two-sided p-values with a level of significance (alpha) of <0.05.

Missing data

For Missing Data, we will consider that 15% of patients would drop out from this study, and thus missing data will be handled with the Last Observation Carried Methods (LOCF) that is when data at endpoint are imputed from the last observation. We also will use the Intention-to-treat analysis (ITT), to preserve the subjects according to the original randomized group in final analysis (Myers, 2000).

The ITT population is defined as all randomized patients with rUTI who received at least one dose of study drug.

Data management

The data will be collected in a paper Clinical Report Form (CRF), then will be transcribed in the electronic data based REDCap (Research Electronic Data Capture). The REDCap is a secure web application for building and managing online surveys and databases; you may input data from anywhere around the world with secure web authentication and modify the database or survey at any time during the study. Data may be imported from external data sources ("REDCap," 2015).

There will be a Core Coordination Center responsible for data coding and data management.

The data access security will be made by the password requirements. The paper CRF will be kept in fireproof locked cabinets. All data will be retained for a period of five years, according to the regulatory requirements ("Data Management," 2015).

For assure the data quality the double data entry will be made and then it will be checked for inconsistencies, unexpected values and missing values.

Data monitoring

The main reason to establish a Data Monitoring Committee (DMC) is to enhance safety of the participants in situations in which safety concerns may be unusually high ("Guidance for Clinical Trial Sponsors - Establishment and Operation of Clinical Trial Data Monitoring Committees," n.d.). Some aspects that should be set up in order to decide whether to establish a DMC are: indication, study endpoints, study duration, study population and available knowledge about the drug ("Guideline on data monitoring committees.," 2005).

The intervention is not intended to prolong life or reduce risk of a major adverse health outcome, it is not invasive and the study is not being performed in a potentially fragile population (such as children and pregnant women) or at elevated risk of death ("Guidance for Clinical Trial Sponsors - Establishment and Operation of Clinical Trial Data Monitoring Committees," n.d.).

A DMC was not established considering the stated above and that the purpose of this study is the prevention of recurrent E. coli UTI.

Additionally, the drug under investigation (Vitamin D) is well characterized and known for not harming patients at study doses.

The safety of the participants will be, nonetheless, monitored by the sponsor and by the investigators, especially concerning overdose of Vitamin D supplementation by pre-scheduled visits and laboratorial tests.

Interim analysis

The interim analyses are generally performed in trials that have a DMC, longer duration of recruitment and potentially serious outcomes. The results of these analyses helps to inform whether the trial should be continued, modified or halted earlier than intended for benefit, harm or futility.

Therefore, considering this trial will not establish a DMC, will not have a longer duration of recruitment or a potentially serious outcome, there will not be established in the protocol any interim analyses or stopping guidelines for this prevention trial.

Additionally, interim analyses might delay the finalization of the trial due to the time needed for the results and statistical analyses.

Ethics: Informed consent

The trial will be carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki, in accordance with the International Conference on Harmonization, Harmonized Tripartite Guideline for

Good Clinical Practice (GCP). Standard medical care (prohylactic, diagnostic and therapeutic procedures) remains responsibility of the treating physician of the patient.

The investigator should inform the sponsor immediately of any urgent safety measures taken to protect the study patients against any immediate hazard, and also of any serious violations of the protocol.

The rights of the investigator/trial site and of the sponsor with regard to publication of the results of this trial are described in the investigator/trial site contract. If an interim report is generated for regulatory approval, data may be published.

This trial will be initiated only after all required legal documentation has been reviewed and approved by the respective Institution Review Board (IRB) / Independent Ethics Committee (IEC) and competent authority (CA) according to National and International regulations. The same applies for the implementation of changes introduced by amendments.

Prior to patient participation in the trial, written informed consent must be obtained from each patient according to ICH GCP and to the regulatory and legal requirements of the participating country. Each signature must be personally dated by each signatory and the informed consent and any additional patient-information from retained by the investigator as part of the trial records. A signed copy of the informed consent and any additional patient information must be given to each patient of patient´s legally authorized representative (LAR). Informed consents will be obtained by the principal investigator or the study coordinator or one designated co- investigator.

Ethics: Confidentiality

Research staff will ensure that the condition under which a procedure is performed or information is collected is protected against interaction with other participants, overheard, intercept or viewed. Patient visit will be assessed in private settings and there will be no use of telephone to answer sensitive questions ("'Confidentiality' SPIRIT, standar protocol items: recommendations for interventional trials.," 2015).

Study related information (laboratory reports, data collection, and administrative forms) will be identified with a unique code for each participant in order to maintain confidentiality. A custom-designed database will store de- personalized patient data; it will use encrypted digital files in a secure password-protected access system only accessible to designated researchers. All data from paper CRFs will be entered by one blinded research office staff and the hard copies that contain names and personal identifiers will be stored in a locked cabinet in a limited access office at each study site. Databases will be stored in a locked cabinet in a limited access office at each study site. A second research office staff will review database every three months and cross-reference with existing medical records, any discrepancy must be explained. This process will be executed at each study site and will have limiting access to the minimum number of individuals necessary for quality control, audit and analysis. The investigators will only have access to the database on completion of the study. Since this is a multicenter study, data will be transmitted to the rest of co investigators with a virtual private network internet password-protected and encrypted system ("'Confidentiality' SPIRIT, standar protocol items:

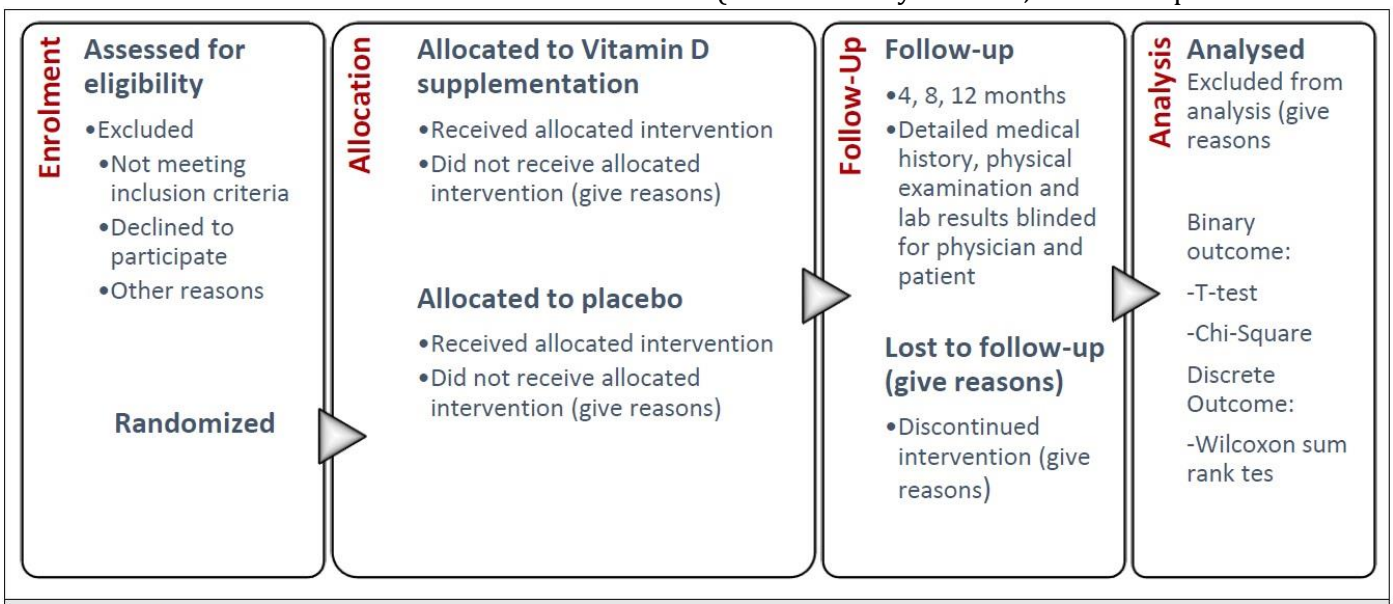

Figure 2. Study outline.

recommendations for interventional trials.," 2015, "'Participant privacy and data confidentiality,'" 2015).

In a situation where there is a legally required release of private information will need notification to patient and authorization documents disclosure. These situations include: elder and dependent adult abuse reporting, sexual assault and rape reporting, warning to police or potential victim when an individual is deemed a danger to others, reporting treatment of person suffering from aggressive or abusive behavior and reporting certain communicable diseases ("'Participant privacy and data confidentiality,'" 2015).

Data quality assurance

A quality assurance audit/inspection of this trial may be conducted by the sponsor or the IRBs/IECs or by regulatory authorities. The quality assurance auditor will have access to all medical records, the investigator's trial-related files and correspondence, and the informed consent documentation of this clinical trial.

The investigator/institution will permit trial-related monitoring, audits, IRB/IEC review and regulatory inspection, providing direct access to all related source data/documents. CRFs and all source documents, including progress notes and copies of laboratory and medical test results must be available at all times for review by the sponsor's clinical trial monitor, auditor and inspection by health authorities (such as FDA). The Clinical Research Associate (CRA) on site monitor and auditor may review all CRFs and written informed consents. The accuracy of the data will be verified by reviewing the documents.

Authorship

From the beginning of the trial, authorship guideline will be disclosed and all investigators should approve them before enrolling in the trial. "An efficient, fair and effective way to establish authorship on study-related manuscripts could diminish conflict among the investigators and help ensure robust and timely dissemination of study results. The HF-ACTION (Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training) trial established authorship-scoring system in an equitable and transparent manner based on objective, quantifiable contributions to the study as a whole. The HF-ACTION investigators developed the scoring system that assigned points to investigators by using the criteria established for enrollment, adherence to the exercise program, data completion, committee service, and other trial efforts." ("Whellan DJ, Ellis SJ, Kraus WE, Hawthorne K, Pina IL, Ketevian SJ; Kitzman DW, Cooper L, Lee K, O'Connor CM. Method for establishing authorship in a multicenter clinical trial. Ann Intern Med 2009; 151:414-20," n.d.).

DISCUSSION

Urinary tract infection primarily affects young women (20-24 years of age)(Betsy Foxman & Brown, 2003). Around 5% of those women who present with an initial UTI will have multiple episodes within a year (Winberg, Bergström, & Jacobsson, 1975) and, although considered to be a benign condition, rUTI can have a significant impact in quality of life. Despite the alternatives of treatments that already exist for rUTI, new strategies for preventing rUTIs are needed. The correction of serum levels of 25 (OH) Vitamin D could prevent the recurrence of UTI, however literature is limited and this study intends to define the role of Vit D for the prevention of urinary infections as well as the incidence.

This will be a multicenter randomized, double blind controlled trial. Target population are women older than 18 years of age with privouse diagnosis of rUTI due to E.Coli. it will be a 2-arm parallel design, allocation is 1:1 to either receive placebo or Vitamin D at a dose of 2000 IU/day, follow up visits during one year every 4 months from initiation date. Statistical analysis will includet-test,Chi-squared and Wilcoxon Sum Rank Test for continuous, binary and discrete outcomes respectively. To assess Vit D effect, risk ratios (RRs) with 95% confidence interval (CIs). Finally an ITT analyses and a second stratified analysis by age will be performed.

Conflict of interest and financial disclosure.

The authors followed the International Committee or Journal of Medical Journals Editors (ICMJE) form for disclosure of potential conflicts of interest. All listed authors concur with the submission of the manuscript, the final version has been approved by all authors. The authors have no financial or personal conflicts of interest.

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