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Study Protocol

Rationale for a Vitamin K2 and Vitamin D3 Intervention Trial in Children and Adolescents with Low-Energy Bone Fractures

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Abstract

Background: The role of vitamin K2, especially menaquinone-7, has recently been highlighted in the literature and distinguished from vitamin K1 in maintaining calcium homeostasis and a healthy skeletal system. Epidemiological and clinical research provides a new nutritional paradigm for efficient and safe delivery of calcium that requires co-supplementation with the fat-soluble vitamins D and K. ***Objective:*** We propose a prospective, three-month, randomized, double-blind, placebo-controlled intervention trial (RDBPC), investigating the effects of vitamin D3 in the presence and absence of vitamin K2 (menaquinone-7, MK-7) on the healing process of low-energy bone fractures in children and adolescents. Thus, information will be obtained regarding the role of vitamin K2 in the bone healing process. ***Methods:*** Ninety pediatric patients with low blood levels of vitamin D and low energy bone fractures will be divided into three groups that receive daily for three-month identical soft gel capsules containing 2000 IU vitamin D3, 90 mcg of vitamin K2 as menaquinone-7 (MK-7) combined with 2,000 IU D3 or olive oil-containing placebo. Patients will visit the clinic on weeks 1, 2, 4, 6, 8 and 12, and will be evaluated by X-ray regarding progress in bone union and range of joint motion. Blood samples will be collected in duplicates on day 0 and after the 3-month regimen. The blood samples will be analyzed for vitamin K (menaquinone-7, MK-7) and vitamin D as well as the bone turnover markers bone-specific alkaline phosphatase (BALP) and N-terminal telopeptide (NTX). ***Conclusions:*** This proposed nutritional regimen will provide new information regarding the ability of vitamins D3 and K (specifically MK-7) in combination therapy to heal and prevent low-energy fractures among children and young adults. It will also contribute to building the "bone bank," therefore helping to prevent the risk of fractures in adulthood and the development of osteoporosis later in life.

ClinicalTrials.gov Identifier: NCT03871322

Keywords: ***low-energy fractures, children, adolescents, vitamin K2, menaquinone-7 (MK-7), vitamin D3, bone markers***

Introduction

In the past decade, an increased interest in the roles of vitamins D and K concerning bone health and prevention of low-energy bone fractures in children has occurred [1-4]. Low-energy fractures, especially fractures of the forearm, are increasingly common in childhood and defined as fractures sustained from a fall from the patient's height or a fall during team sports [1,5,6]. Different

environmental and genetic risk factors and nutritional inadequacies may be contributing to bone fractures in children [1]. Our previously published clinical and epidemiological research highlighted the potential relationship between vitamin D and K deficiency or insufficiency in children and the increased incidence of low-energy bone fractures [7-9].

Vitamin D is involved in the synthesis of osteocalcin, a bone-multitasking protein that is the second most abundant bone protein after collagen [10]. Manufactured by osteoblasts, osteocalcin is responsible for the delivery of calcium to the bone, regulating calcium homeostasis in the bone, stimulating collagen synthesis by osteoblasts, and promoting the build-up of bone matrix [10]. The essential role of osteocalcin may be made possible by vitamin K, enabling it to attach and transport calcium for specific body functions, including maintaining resilient bone in a potential prevention and healing process of bone fractures [11].

In the 2018 published clinical study, we evaluated vitamin D and K in children with low-energy fractures and children without fractures [9]. The status of both vitamins was evaluated based on serum levels of total vitamin D [25(OH) D3 plus 25(OH) D2], calcium, bone alkaline phosphatase (BALP), N-terminal telopeptide (NTX), and the ratio of serum levels of uncarboxylated to carboxylated osteocalcin or UCR which is a sensitive indicator of bone vitamin K status. The study involved 20 children (14 boys, six girls) aged 5 to 15 years old, with clinically confirmed low-energy fractures compared with the control group of 19 healthy children (9 boys, ten girls), aged 7 to 17, without fractures.

While there were no statistically significant differences in the serum calcium, NTX, BALP or total vitamin D levels between the two groups, there was a statistically significant difference in the UCR ratio, indicating a difference in vitamin K status. The median UCR in the fracture group was 0.471 compared with the control group value of 0.245 ($p<0.0001$). Based on regression analysis, this pilot study showed vitamin K status significantly correlated with the lower rate of low-energy fracture incidence [9].

Our study results [9] support epidemiological evidence indicating a disturbing growth in the number of cases of low-energy fractures in healthy children and adolescents. Approximately one-third of all children suffer at least one fracture before 17 years old, most commonly in the distal forearm [12]. Unlike the fractures in adults, pediatric fractures heal more rapidly, have a higher potential to remodel, and need a shorter immobilization period [13]. However, recent evidence suggests that a childhood fracture is a predictor of lower adult bone mass and increased fracture risk in adulthood [14,15].

Although multiple risk factors may contribute to this growing health concern, deficient or insufficient nutrition and nutrients, including calcium, vitamin D and vitamin K are among leading considerations [1]. The particular role of vitamin K2, especially menaquinone-7 [MK-7], has been highlighted in the recent literature and distinguished from vitamin K1 in maintaining calcium homeostasis and a healthy skeletal system [1-4]. The epidemiological and clinical research provides a new nutritional paradigm for efficient and safe delivery of calcium that requires co-supplementation with the fat-soluble vitamins D and K [1]. This new proposed nutritional regimen may prevent low-energy fractures among children and young adults. However, it will also contribute to building the "bone bank," therefore helping to prevent the risk of fractures in adulthood and the development of osteoporosis later in life.

Given the outcome of the 2018 pilot study [9], we are proposing a prospective, three-month, randomized, double-blind, placebo-controlled intervention trial (RDBPC), investigating the effects of vitamin K2 (menaquinone-7, MK-7) and vitamin D3 on the healing process of low-energy bone fractures in children and adolescents (**Figure 1**).

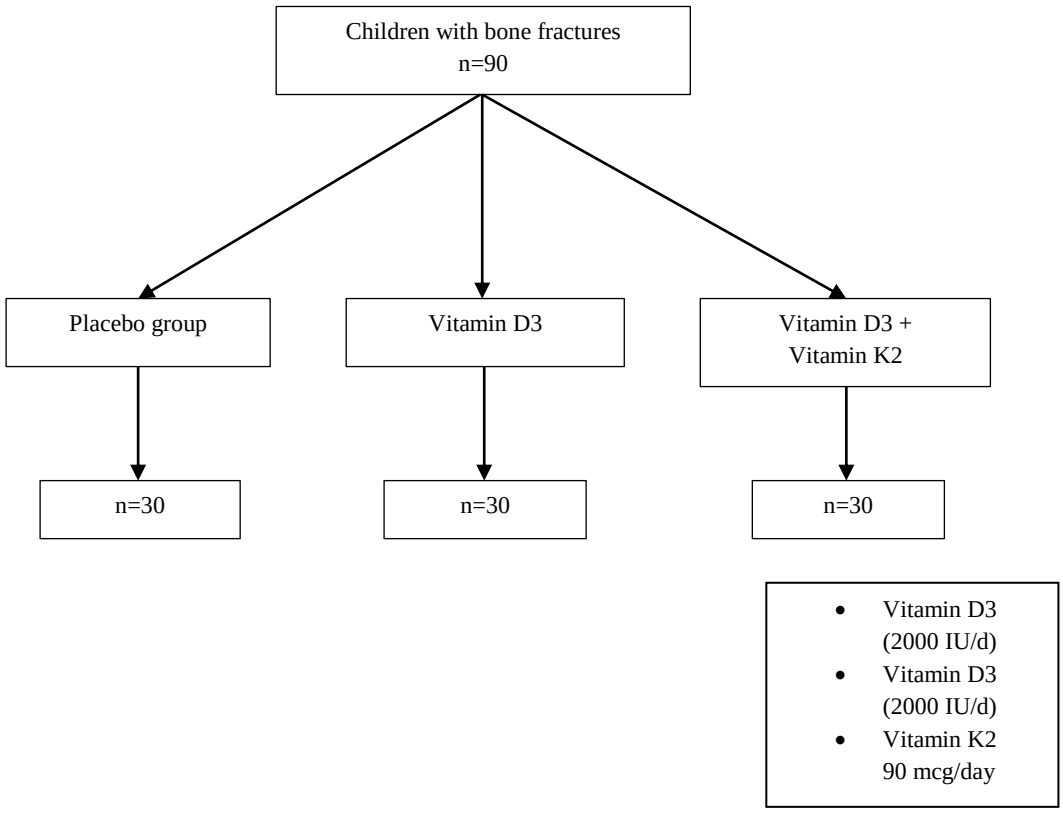

Figure 1: Flow Chart of Supplementation with Vitamins K2 and/or D3 and Placebo in Children with Low Energy Fractures.

Inclusion criteria

- Age < 18 years
- Presence of low-energy fracture
- The level of vitamin D in blood <30 ng/ml

Exclusion criteria

- Age > 18 years
- Lack of low-energy bone fracture
- Oral anticoagulants treatments, which interfere with vitamin K cycle
- Current supplementation with vitamin K2 or vitamin D3
- Osteogenesis imperfecta and other bone diseases

Methods

Subjects

This three-month RDBPC study will involve a group of 90 pediatric patients screened for low-energy fractures at the Department of Pediatric Orthopedics and Traumatology, Medical University of Białystok, Poland, and selected based on the exclusion and inclusion criteria listed in Figure 1. The study team will obtain written informed consent from parents of all children entered into the study and ultimately selected for the study. The Bioethical Committee of the Medical University of Białystok, Poland, has approved the proposed study's merit and design, approval R-1-002/283/2018). The study team has secured the international registration for this clinical study - ClinicalTrials.gov Identifier: NCT03871322.

At the patients' admission to the orthopedic outpatient clinic, the principal investigator (PI) will examine the patient. The pediatric orthopedist will establish low-energy fractures based on the anamnesis, physical examination, and radiological evaluation. Tests to obtain the baseline blood levels of total vitamin D [25-hydroxy vitamin D3 (25(OH)D3) and 25-hydroxy vitamin D2 (25(OH)D2)] will be performed, and only children with vitamin D levels lower than 30 ng/ml in the blood will be included in the study. The normal values for circulating vitamin D, according to our laboratory are 30.0 – 74.0 ng/ml of plasma.

An independent investigator will randomly assign the study population to the three study groups. The groups will receive daily for three-months identical-looking soft gel capsules (1 capsule/patient/day) containing 2,000 IU vitamin D3, 90 mcg of vitamin K2 as menaquinone-7 combined with 2,000 IU D3, or olive oil-containing placebo capsules, respectively. Synergia Ltd. Mumbai, India will prepare and validate the three capsule products, and the University pharmacy will keep the supplements code-labeled in standard room storage conditions until use.

During the 3-month follow-up visits to the outpatient orthopedic clinic, the pediatric orthopedist will examine the patients, evaluate the X-rays, and determine the progress in bone union and the range of joint motion on a blinded basis. All patients will be questioned and examined for any subjective or objective side effects in the course of the study. The patients will visit the clinic on weeks 1, 2, 4, 6, 8 and 12. The University pharmacy will provide the code-labeled supplements to the PI and monitor compliance of taking the supplements by registering a pill count in returned containers by each patient during the scheduled visits.

Analytical Procedures

Blood samples will be collected in duplicates upon admission to the study on day 0 and after the 3-month regimen. The University laboratory will collect blood samples to evaluate bone turnover markers and the status of vitamin K and vitamin D. The primary evaluation end will include: the dynamics of fracture healing, changes in levels of osteocalcin, and vitamin K (MK-7) and vitamin D levels against the placebo group. The study team will evaluate bone fracture healing milestones based on bone union defined as the absence of pain and the presence of bridging callus in three of the four cortices seen on the AP and lateral radiographic views of the bone. Delayed union is defined as incomplete consolidation at 90 days, as Schmittenbecher et al. [16] described. The secondary evaluation endpoints will consist of changes in bone markers, such as bone-specific alkaline phosphatase (BALP), N-terminal telopeptide (NTX), and the evaluation of the range of motion of the fractured limb, using the goniometer and criteria described by Price et al. [17] and Flynn et al. [18].

The blood samples will be processed according to the following procedures [9]. After blood sampling, blood serum samples will be frozen and kept at -80 C until use. Two biochemical markers of bone turnover will be determined, namely, bone alkaline phosphatase (BALP) as a marker of bone formation and cross-inked N-terminal telopeptide of collagen (NTX) as a marker of bone resorption. Both BALP and NTX will be measured by ELISA kits (Wuhan Fine Biochemical Technology Co., Ltd., China).

To estimate bone vitamin K status, uncarboxylated (ucOC) and carboxylated (cOC) fractions of osteocalcin in serum will be measured. Determination of ucOC and cOC will be performed by ELISA Kit (TAKARA BIO INC., Japan) [9]. All assays will be performed in duplicate. The UCR (ratio between ucOC and cOC) will be calculated as a sensitive indicator of bone vitamin K status. Serum concentrations of total vitamin D [25(OH) D3 and 25(OH)D2] in the study population will be performed by electrochemiluminescence utilizing paramagnetic particles coated with streptavidin and ruthenium compound on the Cubase 411 apparatus by Roche [9].

Statistics

The study data analysis will be performed with a Nonparametric Mann-Whitney test to compare the placebo and treatment group parameters. Statistical significance will be determined between sets of data ($p<0.05$) using a computer software program. Median and quartiles will be used for presentation characteristics of analyzed parameters.

Results and Conclusions

This study is a proof-of-concept trial that will provide information on the potential role of supplementation with vitamin D3 plus vitamin K2 [MK-7] or vitamin D3 alone in the healing process of low-energy bone fractures. Secondarily, additional information will be obtained on the roles of these two vitamins in healing low-energy bone fractures, and the mechanisms involved. This trial can open new therapeutic strategies for bone fracture treatment in children and adolescents, a common injury during childhood and the leading cause of pediatric injury-related visits to the emergency room.

Acknowledgements

JP, KM, MK, and VB are responsible for study design and methodology. JP, TG, and MK will be responsible for patient recruitment. DMS will be responsible for capsule preparation and analysis. SC will perform biochemical analyses. MK, KM, SC, VB

and SJS will analyze results. SJS, MK, KM and VB will write study results and final manuscript. All authors have read and approved this study protocol and manuscript.

Conflict of Interest

The authors declare that they have no conflicts interest.

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