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Original article

Micronutrients and the risk of hip fracture: Case–control study

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SUMMARY

Background: Vitamin D, and possibly vitamin K, has an established association to fracture risk. Other vitamins are, however, less studied.
Aim: To determine whether specific micronutrients other than 25(OH)D and vitamin K play a role in risk of hip fracture and bone turnover.
Methods: In this case–control study, blood was drawn for measurements of vitamins A, B6, B12, C, E, and folic acid as well as the bone turnover markers osteocalcin and bone-specific alkaline phosphatase upon admission for hip fracture in 116 patients and in 73 home-dwelling non fractured controls. Results for vitamin K1 and 25(OH)D from the same populations have been reported previously.
Results: Low vitamin A, C, and E concentrations were independently associated with a risk of hip fracture. The adjusted odds ratio (95% confidence interval) per 10 μmol/L increase in vitamin A concentration was 0.74 (0.65–0.84); for 1 μmol/L vitamin C and E: 0.94 (0.92–0.97) and 0.81 (0.74–0.89) respectively. The results were principally unchanged when 25(OH)D, vitamin K1, Body Mass Index, and other potential confounders were adjusted for. All vitamins except B12 and folic acid correlated positively with total osteocalcin and negatively with bone-specific alkaline phosphatase.
Conclusions: Low vitamin A, C, and E concentrations are associated with an increased risk of hip fracture, possibly mediated through bone turnover mechanisms.
This case–control study is registered at: ClinicalTrials.gov. NCT01738776.
The patient related outcome is also registered at: ClinicalTrials.gov. NCT01009268.

1. Introduction

Low BMI is a well-established risk factor for hip fracture. However, whether a low BMI in itself or a decrease in specific micronutrients leads to the increased risk of fracture is unknown. Bone mass depends on the rate of bone turnover and ability to mineralize newly formed bone. A high rate of bone formation, as measured by bone-specific alkaline phosphatase (BALP), is often found in osteoporotic patients. A high rate of bone formation combined with low active osteocalcin (OC), a Ca-binding protein, may lead to immature bone with insufficient calcification, and may be detrimental for bone health and increase fracture risk.

We previously reported that low vitamin K1 and low 25(OH)D synergistically increase the risk of hip fracture [1]. However, vitamin K1 and 25(OH)D were poorly correlated with total osteocalcin (totOC) and BALP, and only a minor fraction of the variance in totOC and BALP were attributable to vitamin K1and 25(OH)D concentrations.

Vitamins C and E and the vitamin B family have received attention as potentially protective agents against degenerative disease, including osteoporosis [2]. A high intake of fruits and vegetables as well as low homocysteine is reported to be associated with a lower risk of hip fracture [3]. Whether this is a direct action

Abbreviations: BADL, barthel activities of daily living; BALP, Bone-specific alkaline phosphatase; BMD, bone mineral density; CRP, C-reactive protein; HGS, hand grip strength; OR, odds ratio; OC, osteocalcin; OOT, Oslo Orthogeriatric Trial; PTH, parathyroid hormone; totOC, total osteocalcin; ucOC, undercarboxylated osteocalcin.

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of vitamin C, vitamin E, or vitamin B, other nutrients, or a more indirect association with fruit and vegetable intake as a marker for a healthier lifestyle, is unknown.

Several possible protective mechanisms of action of vitamins C and E on hip fracture have been suggested, such as their ability to reduce oxidative stress and inhibit bone resorption. Moreover, vitamin C is necessary for the synthesis of collagen, and is important for bone quality and tensile strength [4]. In addition, deficiencies in vitamin B (B6, B12, and folic acid), along with the consequent elevation in homocysteine, are suggested to be detrimental to bone [5–10]. Epidemiological studies have found that excessive vitamin A intake is a possible risk factor for hip fracture [11–13]. However, other epidemiological studies have failed to confirm this association [14]. It has been suggested that vitamin A may inhibit bone resorption and perhaps stimulate bone formation as well and possibly act in synergy with vitamin D in bone mineralization [15].

Therefore, the aims of this case–control study were to determine whether low serum concentrations of vitamins A, C, E, and B (B6, B12 and folic acid) are associated with an increased risk of hip fracture in aged patients, if a potential association is independent of 25(OH)D, vitamin K1 and general malnutrition, and to examine whether a potential association can be explained through the bone turnover markers OC and BALP.

2. Material and methods

2.1. Study population

2.1.1. Cases

This case–control study was a substudy of the Oslo Orthogeriatric Trial (OOT) [16]. The patients (cases) in the OOT were consecutively admitted to Oslo University Hospital, Ullevål, Norway. Inclusion criteria in the OOT were those acutely admitted for a hip fracture as result of a low energy trauma, defined as a fall from 1 m or lower. Exclusion criteria was patients who were regarded as moribund at admittance (as determined by the admitting orthopaedic surgeon based upon their clinical experience) and patients lacking a valid informed consent or assent.

The study population was examined during September 2009 to April 2011 for the nutrition sub study. Of 216 eligible patients enrolled in the OOT, 116 patients had a preoperative blood sample for vitamin analysis purposes performed and could be included in the case control study. Missing cases were due to that the patient was operated for hip fracture before there was time for blood drawl for the nutrition sub study, low capacity to draw blood for project purposes at weekends, holidays, and at night. For technical reasons, we were not able to obtain all vitamin analyses in all patients. Thus, analyses are missing for: vitamin A, $n = 10$; vitamin C, $n = 28$, vitamin E, $n = 8$; vitamin B6 $= 8$, vitamin B12 $= 12$ and folic acid, $n = 14$.

Baseline data for the 216 patients included in the OOT were registered and all underwent a pre-surgical blood test as part of the clinical routine. There were no differences in age, BMI, morbidity, C-reactive protein (CRP) or albumin, between those enrolled for vitamin and bone turnover tests and those not enrolled (data not shown).

2.1.2. Controls

The control group consisted of individuals with no previous hip fracture, and was drawn at random by Statistics Norway from home-dwelling inhabitants aged 60–100 years (median age, 82 years) in the census files of Oslo in 2005. The control subjects were contacted by mail and followed up by two phone calls. A total of 73 control subjects (66% women) were recruited.

The catchment area for the cases and controls was the city and suburbs of Oslo, Norway.

2.2. Data collection

Designated project staff collected the data in cases and controls. In cases, weight was measured using a class 3 chair scale as soon as possible post-surgery. Patients wore light clothing. Height was either measured using a tape measure against a wall or calculated from measured knee height [17]. The knee was flexed so that it was bent at 90° and measurements were taken from the anterior surface of the thigh near the patella to under the heel. Residence status was registered as home dwelling or institutionalized; alcohol consumption in total abstainers and non-abstainers, and smoking habits in current smokers or non smokers. The number of prescription medications used was recorded. Activity of daily living was measured using the Barthel Activities of Daily Living (BADL) Index [18]. Handgrip strength (HGS) was examined by hand dynamometry (Jamar, Germany: three repetitions per examination) in the dominant arm. Patients were examined daily preoperatively and until the fifth postoperative day. The best handgrip test was used.

In the controls, weight was measured using a class 4 standing scale, and they wore light clothing. Standing height was measured with a tape measure towards a wall. Smoking and alcohol consumption were recorded and categorized as for the patients. The number of medications was recorded and the same BADL form was used for measuring daily activity. For HGS, the best result of three repetitions was used.

2.3. Preparation and analysis of blood samples

In patients, blood was collected by venipuncture shortly after admission for hip fracture prior to the operation. In controls, blood was collected in the morning by venipuncture following an overnight fast. All samples were clotted for 30 min at room temperature and serum was separated by centrifugation. Aliquots were immediately stored at −80 °C, and later analyzed for serum vitamins A, C, E, B6, B12, and folic acid. The bone turnover markers totOC, BALP, and parathyroid hormone were assayed in serum.

Analysis of vitamin C was performed within 14 days after sampling, according to Zannoni et al. [19]. Vitamins A, E and B6 were continuously analyzed within 2 months after sampling. Vitamin B12, and folic acid were analyzed in one batch at the end of the study.

Laboratory assays of retinol (vitamin A) were performed using the Bio-Rad Laboratories kit (Munich, Germany). Alpha-tocopherol (vitamin E) was assayed by radioimmunoassay also from Bio-Rad Laboratories. High pressure liquid chromatography was used for assays of pyridoxal-5'-phosphate (vitamin B6) by Chromsystems (Munich, Germany). Folic acid and cobalamin (vitamin B12) were assayed with a Hitachi 717 Modular multianalyzer (Boehringer Mannheim, Germany). Analysis of vitamin K1, 25(OH)D, and bone turnover indicators was performed as described previously [1]. BALP was quantified in E/L with 1E = 1 μmol hydrolyzed p-NPP/min (p-NPP is a monoclonal anti-bone-ALP antigen). Vitamins C, E, and A, and the B vitamins were analyzed in the Department of Clinical Chemistry or Nutrition laboratory, department of Medical Biochemistry at Oslo University Hospital, Norway.

The coefficients of variation for the analyses of the vitamins A, C, E and B6 ranged from 2.5% to 4.5%. The coefficient of variance for vitamin B12 and folic acid was 6.8 and 9.7 respectively. These coefficients of variation remained stable over time. None of the methods were changed and we used the same laboratories for cases and controls during the entire project period.

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