ATYPICAL FEMORAL FRACTURES - SINGLE CENTER DATA

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Background: Atypical femoral fractures (ATF) have drawn much attention during the last years, especially in light of their possible connection to bisphosphonate use. Reported cases have been reviewed and position statements of ASBMR and the Endocrine Society have been published with sets of criteria for more precise case definition. Our aim was to review the prevalence, clinical and radiological parameters of patients with ATF at our institution.

Methods: Computerized database of discharge diagnoses (2009-2010) was reviewed. ICD-9 diagnoses compatible with the location of the fracture below femoral neck were chosen (e.g.: Shaft, Supracondylar, Subtrochanteric, etc). Patients younger than 50 years old and those with major trauma were excluded. Admission femoral X-rays were examined by a senior radiologist. The fractures were classified as ATF or not-atypical according to the published criteria. Hospital files of patients with ATF were reviewed.

Results: Our hospital cares for 300 patients with femoral fractures annually. Forty-two patients answered the search criteria. Of those, only 16 (2.5%) were classified by the radiologist as having an ATF.
The diagnostic codes used in patients with ATF were: "shaft fractures" (62.5%), "supracondylar" (18.7%) and "subtrochanteric" (18.7%). Most fractures coded as "subtrochanteric" were intratrochanteric, and thus, were excluded. Fourteen were women, age 72±12, 52-94.
The average hospital stay was 7.9±3.2 days. Five patients were functionally intact prior to fracture, the vast majority were frail–bedridden, psychiatric ward inpatients, or dependent in ADL. Two patients received PPIs, two patients received alendronate and one received both. None were on current steroid treatment.

Conclusions: ATF are not uncommon. In our analysis, 2.5% of all femoral fractures were atypical. Lack of uniform code designation makes the case identification difficult. Most patients with ATF are frail. Less than third (5/16) of our patients were exposed to medications linked to increased risk for ATF. Uniform code for ATF is needed to allow data collection.

INHIBITION OF OSTEOCLAST DIFFERENTIATION BY CAROTENOID DERIVATIVES

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Several epidemiological studies indicate that consumption of fruit and vegetables has a beneficial role in bone health. Bone remodelling, an essential process for bone health is mediated by osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells). The goal of this study is to determine if various phytonutrients inhibit osteoclast differentiation and by what mechanism. Our experimental system is based on induction of differentiation of the monocyte/macrophge murine cell line- RAW264.7 to osteoclasts by Receptor Activator of NF-κB Ligand (RANKL). ROS stimulate osteoclast differentiation by increasing RANKL production in cells of the stromal/osteoblastic lineage as well as augmenting the NF-κB mediated differentiation mechanism in osteoclast precursors. Osteoclast differentiation was measured by a quantitive assay for Tartrate Resistant Acid Phosphatise (TRAP) and by counting osteoclasts as TRAP positive multinucleated cells.

Differentiation was inhibited by various phytonutrients such as the carotenoid lycopene and its derivatives, the polyphenols carnosic acid, resveratrol and curcumin and the isothiocyanate sulforaphane. Our previous studies have shown that oxidized derivatives of carotenoids are mediating some of their biological effects. For example, activation of the transcription factor Nrf2, in cancer and osteoblast cells, was caused by aldehyde derivatives of carotenoids, probably through interaction with thiol groups in the inhibitory protein Keap1. Thus, we analyzed the structure-activity relationship of a series of dialdehyde carotenoid derivatives in inhibition of osteoclast differentiation. We found that the degree of inhibition by these derivatives depends on the distance of the methyl group from the terminal aldehyde, which determines the reactivity of the conjugated double bond in reactions such as Michael addition to thiol groups in proteins. Moreover, the carotenoid derivatives attenuated the NFκB signal through inhibition of IκB phosphorylation (western blot).

In conclusion, various phytonutrients inhibit osteoclast differentiation. The effect of the carotenoid derivatives on this system is mediated, at least partially by inhibition of the NF-κB pathway.

POST FRACTURE OSTEOPOROSIS TREATMENT PROGRAM, IS IT EFFICIENT?

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Objectives: To assess effectiveness of hospital based Fractures Prevention Program (FPP) in the fracture prevention treatment in patients with previous fragility fractures.

Patients and methods: FPP was initiated in Rambam Health Care Campus in March 2009. All patients with fragility fractures were referred from the Department of Orthopedic Surgery to the Bone and Mineral Metabolism Unit for fractures prevention treatment.

Results: 900 patients, aged 46-107 (75.18±11.7), 247 (27.4%) men, 653 (72.6%) women were hospitalized with fractures since March 2009: 593 (66%) for hip fractures, 60 (7%) for vertebral fractures, 247 (27%) for other fractures. 155 (17%) had previous fragility fractures. Prior to hospitalization 152 (23.2%) women have received a fracture prevention treatment: 134 (88.2%) with oral bisphosphonates (114 – alendronate, 20 - risedronate), 10 (6.5%) with raloxifen, 5 (3.3%) with teriparatide. Four (1.6%) men were treated before hospital admission with alendronate. 25OHD serum levels prior to hospitalization were available for 239 (26.5%) patients. Mean 25OHD was 26.5±14.7 (4-118) ng/ml; 85 (35.6%) patients had vitamin D deficiency (25OHD ≤20 ng/ml), 25 (10.5%) - severe vitamin D deficiency (25OHD ≤10 ng/ml).
154 (17.1%) patients, 23 (9.3%) men, 131 (20.1%) women, adhered to the FPP clinic visits: 98 (63.6%) had femoral neck fractures, 56 (36.4%) – other fractures. 746 (82.9%) patients stayed out of the FPP: 52 (10.6%) women and 1 (0.3%) men are treated in the community, 601 (80.4%) remain untreated,18 (2.4%) died, 74 (10%) lost to follow-up. 165 (18.3%) patients are currently treated for osteoporosis in the FPP: 80 (48.5%) receive alendronate, 46 (27.9%) – risedronate, 5 (3%) – raloxifen, 32 (19.3%) – zoledronate, 28 (16.9%) - teriparatide, 16 (9.6%) – calcium and vitamin D prior to starting bisphosphonates. 53 patients are treated in the community.

Conclusion: Majority of the elderly patients remain untreated after fragility fractures. Men and hip fractures patients are more likely to remain untreated. Hospital based FPP increased by 10% the rate of FPT.

VITAMIN D INCREASES THE EXPRESSION OF FAK IN KERATINOCYTES LEADING TO ACCELERATED MIGRATION

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Introduction: The migration of epidermal keratinocytes is a critical element in the re-epithelialization phase of wound healing. Epidermal keratinocytes contain an autonomous vitamin D endocrine system, capable of synthesizing the hormonal form, calcitriol, degrading it and responding to it. We have previously shown that calcitriol accelerated the closure of the gap in an in-vitro scratch model.

Objective: to explore the effect of the hormone on the migration process and the mechanism of its motogenic action.

Methods: We used the immortalized non-tumorigenic HaCaT keratinocytes, which are thought to represent the population of basal keratinocytes in the absence of exogenous growth factors or active ingredients. We developed a "Scatter Assay" in which the dispersion of keratinocyte aggregates was monitored by phase and time-lapse microscopy. We developed a "multiple scratch assay" method to compare protein and mRNA levels in migrating and resting cells. The levels of proteins (activated or total) were determined by western blotting and GST pull down assay and the levels of mRNA were determined by real-time PCR.

Results: 24 hour pretreatment of HaCaT cells with calcitriol, significantly and consistently accelerated the migration of HaCaT cells. The effect of the hormone was dose dependent, already apparent at a concentration of 10 nM. The average speed of migration and the straightness of movement were significantly higher in calcitriol treated cultures. The effect on the migratory apparatus is manifested by the activation of the small G protein Rac1. By using pharmacological inhibitors we excluded the involvement TGFβ HGF and cathelicidin as mediators of calcitriol action. We found that pretreatment with calcitriol increased the protein and mRNA levels of Focal Adhesion Kinase, FAK, that is known to play a pivotal role in cell migration. This leads to increased levels of activated FAK in migrating cells.

Conclusions: We conclude that treatment with calcitriol as a single agent prepares the keratinocyte for accelerated migration that may contribute to re-epithelialization during cutaneous wound healing.

BONE GLA PROTEIN INDUCES CARTILAGE AND VASCULAR CALCIFICATION VIA HIF1α-DEPENDENT GLUCOSE METABOLISM

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Vascular calcification arises from a phenotypic transformation of vascular smooth muscle cells (VSMCs) into osteochondrocytic-like ones. Bone Gla Protein (BGP, osteocalcin) is commonly present in the calcified vasculature. Although the role of BGP in calcification is unclear, it was recently reported to act as an energy metabolism-regulating hormone. This study investigates the role of BGP in glucose metabolism and in cartilage and vasculature mineralization.

We established an *in-vitro* BGP-overexpression model in chondrocytes (ATDC5) and VSMCs (MOVAS). BGP overexpression stimulated chondrogenic differentiation and mineralization, increasing the expression of Sox9, Runx2, collagen type X, and staining for alkaline phosphatase, proteoglycans and mineral deposits in both ATDC5 and MOVAS cells. In addition, BGP overexpression enhanced glucose uptake and cell proliferation. Over the course of differentiation, BGP overexpression increased the expression of glucose transporters and key glycolysis enzymes, while downregulating gluconeogenesis enzymes. Both BGP overexpression and treatment with purified BGP resulted in stabilization of hypoxia-inducible factor 1α (HIF-1α) in both cell types, shown by silencing using HIF-1α siRNA to be essential in mediating the direct metabolic effect of BGP. The *in-vivo* model of $1,25(OH)_2D_3$-induced vascular calcification in young rats supported the *in-vitro* observations, showing a correlation between calcification, elevated BGP levels and increased HIF-1α expression in aortae and bone growth plates.
This study demonstrates a novel mechanism by which BGP locally shifts cells toward glycolytic breakdown of glucose, in a HIF-1α-dependent manner, and stimulates calcification of cartilage and vasculature.

VITAMIN D LEVELS IN PEDIATRIC PATIENTS WITH MALIGNANCY

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Background: Multiple studies demonstrated an inverse association between vitamin D and its metabolites and cancer morbidity and mortality. Despite this impressive body of evidence, only a handful of studies estimated vitamin D status in pediatric patients with malignancy. We aimed to assess vitamin D status in a large cohort of pediatric cancer patients and survivors, and to define risk factors to vitamin D deficiency.

Methods: 25OHD levels were obtained in 116 consecutive patients (aged 12.1±6y, M=56) during their routine visits to the hemato-oncology department (mean time from diagnosis 4.13±3.8y). Patients or their parents were asked to answer a questionnaire regarding calcium intake and sun exposure habits.

Results: Average daily calcium intake was 783.6±476mg/day. Mean 25OHD levels were 21.9±9.1ng/ml. Eighteen patients (15.5%) were vitamin D deficient (<11ng/ml), and another 87 (75%) were vitamin D insufficient (11-32ng/ml). Only 11 patients (9.5%) were vitamin D sufficient. Younger age and the amount of sun exposure were associated with higher serum 25OHD levels (r=-0.25, p=0.007; r=0.29, p=0.02, respectively). No association was found with sun protection habits, calcium intake, disease type, gender, years since diagnosis, or undergoing SCT.

Conclusions: The prevalence of vitamin D deficiency and insufficiency in pediatric hemato-oncology patients is high, while daily calcium intake is significantly lower than the RDA. While these values may be similar to those of the general pediatric population in Israel, they are of particular concern in this patient population, which is at high risk for osteoporosis. Furthermore, given the current knowledge regarding the importance of vitamin D in the context of malignancy, maintaining an adequate vitamin D status may be important for recovery and prevention of recurrence of pediatric malignancy.

NUTRITION-INDUCED SERUM FACTOR AFFECTS MIRNAS LEVELS IN THE GROWTH PLATE

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Postnatal skeletal growth takes place in the cartilaginous growth center of the long bones, the epiphyseal growth plate (EGP) and is a genetically determined biological process that is modulated by environmental factors such as nutrition. The local and systemic mechanisms by which nutrition affects growth at the EGP are still not entirely elucidated. MicroRNAs (miRNAs) are small endogenous RNAs that regulate target mRNAs by binding to their 3'UTRs and were shown to be involved in a variety of functions, including skeletal development.

To study the nutrition-growth bond, pre-pubertal rats were subjected to 10 days of 40% food restriction (FR), followed by a renewal of the regular food supply (catch up; CU). A dramatic reduction in EGP height was observed in the FR group, followed by an instantaneous increase after restriction removal. Gene expression pattern was affected as well as several miRNAs.

To identify the mediator between the nutritional status and growth, serum derived from the three groups (control (AL), FR or CU), was added to the culture medium of the chondrocyte cell line, ATDC5, instead of the fetal calf serum. Proliferation was significantly reduced (by 15%; $p<0.05$) in the presence of FR serum compared to AL. One day of refeeding was enough to correct this effect (AL vs. CU; NS). A significant reduction in the miRNAs observed in vivo was also noted in vitro (AL vs. FR; $p<0.05$), followed by an increase with serum of the CU group.

These results are the first to show that miRNA respond to nutritional cues. It also implies the presence of a systemic mediator. Understanding the pathways involved in the transition from quiescence to proliferation in the EGP may lead to a better understanding of the children's growth process, and enable improved manipulation of growth in normal children as well as in those with special nutritional needs.