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The Fingernail Protein Content May predict Bone Turnover in Postmenopausal Women

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Abstract

Background: Bone quality is a relatively new concept that seems to be able to fill the gaps we encounter in the prediction of osteoporosis by bone mineral densitometry. The aim of this study was to investigate relationship between finger nail protein and bone turnover in postmenopausal women.

Methods: In a case-control study 123 postmenopausal women recruited from out patient clinic of Endocrinology and metabolism research center of Tehran University of Medical Sciences. In all participants DEXA scanning and spinal X-ray radiography were performed. Serum Osteocalcin and Cross laps concentrations were measured. Protein extraction from fingernail performed to evaluate protein content.

Results: Fingernail protein content significantly correlated with serum Cross laps concentration (P= 0.03, r= -0.27), lumbar spine BMD (P= 0.01, r=0.4), and total hip BMD (P= 0.01, r= 0.33). In logistic regression analysis, fingernail protein content predicted vertebral fracture (P= 0.002). This relationship was independent of age, BMI, lumbar spine BMD, and total hip BMD.

Conclusion: Common pathways may involve in structural protein synthesis. Thus evaluation of fingernail protein allows an estimation of bone quality, which would lead to a more complete evaluation of bone health.

Keywords: *Fingernail protein, Osteoporosis, Bone turnover*

Introduction

Bone quality is a concept that seems to offer a solution for better prediction of osteoporosis. There is a classic inconsistency about bone mineral density (BMD) and osteoporosis; while low BMD values are associated with increased relative risk of fracture at the population level, the predictive value of BMD in an individual patient remains quite marginal (1-4). Inclusion of increased bone turnover in prediction models has somewhat predicted fracture risk more precisely in favor of bone quality significance (5-9).

In spit of that DEXA scanning is the current gold standard for diagnosis of osteoporosis, its ability to detect individuals who will experience fracture is limited (10). In other words, BMD shows a strong correlation with whole bone strength in the laboratory setting (11), but not in the clinical setting. Only 0% to 44% of the various fragility fractures can be explained by low BMD (4).

BMD measures only the density of bone mineral but not the bone microarchitecture, mineral organization quality, geometric quantities, trabecular structure, microcracks healing capability, and the bone proteins formation (12-15). In fact, BMD is only an indicator of one risk factor for fractures; about 85% of the contribution to the fracture risk is unrelated to BMD (4, 16). Thus bone quality measurement may be helpful for better risk assessment and fracture prediction.

The strength of bone is determined by its elements composition and their structure. (17) Bone must be stiff and also able to resist against deformation. To achieve this resilience it is composed of type I collagen stiffened by crystals of calcium hydroxyapatite. (18). Feature occurrence means that the bone can not provide the contradictory needs: stiffness/flexibility and lightness/ strength (19). An increase in tissue mineral density increases the stiffness of the fabric but sacrifices flexibility so make the bone vulnerable to the fracture though the BMD suggests incongruous condition (17, 20).

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Evidence indicates an important role for changes in collagen content and structure in bone resilience. (21) In normal bone collagen pays for absorbing energy through bone preventing fracture (22). So the composition and degree of collagen cross-linking also influence bone quality (23-26). Since collagen synthesis, secretion, and deposition are matched up with other structural proteins synthesis (27) it may helpful to find a way to appraise the bone matrix quality based on other proteins in other tissues.

Bone collagen and nail keratin are two distinct structural proteins, and both require protein sulfation and disulfide bond formation via cysteine, for structural integrity. Anarchy of either process may lead to disordered collagen and keratin synthesis (27).

The relationship between nail and bone may exist in a measurable way. In the some experience, evaluation of fingernails fragility has been showed as a means for assessing bone health (28). These reports indicated that changes in the organic phase of bone are reflected in similar proteins, such as keratin, from which fingernails are composed. The aim of this study is to investigate relationship between finger nail protein and bone turnover in postmenopausal women.

Material and Methods

Study design and patient population

In a case-control study 123 postmenopausal women recruited from out patient clinic of Endocrinology and metabolism research center (EMRC) of Tehran University of medical sciences. The women were selected consecutively if they fulfilled the criteria and if they were willing to participate in the study. After interview, a general physical examination by a physician was conducted and informed consent was acquired. Blood samples were drawn and centrifuged for 30 min Samples were frozen at -80 ºC in the hormone laboratory of the Endocrinology and Metabolism Research Center. The study protocol was approved by the research ethics committee of EMRC.

Questionnaire

The questionnaire administered at baseline contained questions on demographics, medical history, fracture history, gynecological information, physical activity, and lifestyle variables. To assess fracture history, participants were asked if they had ever suffered from a broken bone, and if so, to give details on which bone, age at first fracture, and level of trauma experienced. The fracture type choices given were vertebral, hip, rib, forearm, and other. Daily intake of dietary calcium and vitamin D was calculated from a food frequency questionnaire that was approved by the nutrition group of EMRC.

Nail sample and Proteins Extraction

A finger nail clipping was taken from each patient where the free edge of the nail plate ends, which contains high sulfur keratin, typical of hard keratins (10).

Human finger nail was washed with ethanol; external lipids were removed using a mixture of chloroform/methanol (2: 1, v/v) for 24 h. Protein extraction was perform with Shindai method as previously describe (29). As brief, the delipidized nail was mixed with a solution (5 ml) containing 25mM Tris–HCl, pH 8.5, 2.6 M thiourea, 5 M urea and 5% 2-mercaptoethanol (2-ME) at 50 °C for three day.

The mixture was filtered and centrifuged at 15000 RPM for 20 min at room temperature. The obtained supernatant was used as a nail protein fraction. The pellet was recovered, washed with distilled water and used as an extracted nail sample. The total protein concentrations of extracted nail samples have been measured by using a visible absorption spectrophotometry method (Hitachi 902 Autoanalyser) with accuracy comparable to standard clinical chemistry methods. In this measurement, within CV run precision was 0.9%-2.3% and between CV run precision was 2.97%- 3.6%.

Measurements

Markers of bone formation included osteocalcin (OC). OC was measured by immunoassay (ELISA) using a Bioscience kit (Nortic Bioscience Diagnostic A/S, Denmark). The intra- and inter-assay CV were 2.6% and 4.7%, respectively. Another marker of bone resorption is the serum C-termi-

nal telopeptides of type I collagen: serum crosslaps. Crosslaps were measured by ELISA using a Bioscience kit (Nortic Bioscience Diagnostic A/S, Denmark), with intra- and inter-assay CV of 5.1% and 6.6%, respectively.

Serum Osteoprotegerin was measured by ELISA using a Immunodiagnostic kit. The intra- and inter-assay CV were 6.6% and 5.7%, respectively.

Serum sRANKL was measured by ELISA using a Biomedica kit, with intra- and inter-assay CV of 4.1% and 5.1%, respectively.

Spinal radiography

Radiograph images were taken by a professional X-ray technician using standard, proven safety precautions.

Lumbar radiographs in the antero-posterior and left lateral projections were acquired following a standardized protocol (30). For the lateral views, subjects were positioned in their left side with knees and hips flexed. Tube-to-film distance was set at 115 cm and films were centered at L3 for lumbar views.

The spinal radiographs were assessed independently by two expert observers (who were both medically qualified) for evidence of osteoporotic vertebral fracture.

BMD measurements

Using DPX Lunar, postero-anterior scans of the lumbar spine (from L1 to L4) and left hip were also acquired to measure BMD. On the basis of their bone mass, patients were classified as normal, osteopenic or osteoporotic, according to the WHO criteria (31).

Visual semiquantitative assessment (SQ)

Conventional radiographs were examined first for quality and then for fractures by an experienced radiologist. According to Genant et al. (32), reductions in the anterior, middle or posterior vertebral heights were classified as mild (20-25% reduction), moderate (25-40% reduction), or severe (>40% reduction).

Statistical analysis

Data were analyzed by means of a personal computer implemented with dedicated software (SPSS 11.5), to obtain mean±SD values, correlation matrix, Student's t-test, analysis of variance and/or χ^2 tests, as appropriate. The level of significance was settled at <5%, as usual.

Results

Totally 123 postmenopausal women in three groups were recruited in the study. All participants based on osteoporosis status and vertebral fractures were classified in three groups that included healthy women (41 women), osteoporotic patients without fracture (42 patients) and patients with fracture (40patients).

The baseline characteristics and BMD (g/cm2) are outlined in table1. There were no significant differences in age, menarche age, and body mass index between three study groups. Healthy women had higher serum Cross laps comparing to osteoporotic and fracture groups. Also BMD of healthy women in all sites were higher than other two groups. Fingernail protein content in patients with vertebral fracture was significantly lower than other two groups. (Fig.1)

Using Pearson's correlation, age was significantly negatively associated with lumbar spine BMD (P= 0.002, r=-0.28), and total hip BMD (P= 0.02, r= -0.2). Similarly, body mass index (BMI) was significantly positively associated with BMD (P= 0.007, r= 0.28), and total hip BMD (P= 0.001, r= 0.35).

Fingernail protein content significantly correlated with serum Cross laps concentration (P= 0.03, r= -0.27), lumbar spine BMD (P= 0.01, r=0.4), and total hip BMD (P= 0.01, r= 0.33). (Fig. 2)

In logistic regression analysis, fingernail protein content predicted vertebral fracture (P= 0.002). This relationship was independent of age, BMI, lumbar spine BMD, and total hip BMD.

Cutoff estimation of fingernail protein content to predict fracture by using ROC curve show that protein content equal 7% may predict fracture with a sensitivity and specificity equal than 87.5% and 68.3% respectively.

In other word, prevalence of patients with fingernail protein content lower this cutoff point was three times higher than healthy women (P=0.001) and Odds ratio in this cross table was 3.13 (95% CI: 1.92-5.02). But this cutoff point could not predict osteoporosis.

Table 1: Measurements data of study population with respect to osteoporosis and fractures

Characteristic	Healthy	Osteoporotic without fracture	Patients with fracture	*P* value ANOVA
Age(years)	53.78 ±6.43	56.14 ±8.47	56.32 ±7.79	0.24
BMI(Kg/m^2)	28.87 ±4.56	27.63 ±4.1	27.33±5.58	0.42
Menarche age(years)	13.43 ±1.19	13.3±1.7	13.53±1.38	0.82
Hip BMD(gr/cm^2)	0.98±0.14	0.83±0.11	0.84 ±0.1	0.001
Spine BMD(gr/cm2)	1.16±0.18	0.92±0.13	0.97±0.15	0.001
Serum Cross Laps (ng/mL)	0.49 ± 0.24	0.7 ±0.45	0.81 ±0.32	0.001
Serum Osteocalcin (ng/mL)	12.41 ±7.47	12.98 ± 9.92	16.55 ±7.42	0.059
Fingernail protein content (%)	10.65 ±8.09	7.06 ± 3.19	4.01 ±2.66	0.001

Values are expressed as mean±SD
ANOVA, analysis of variance

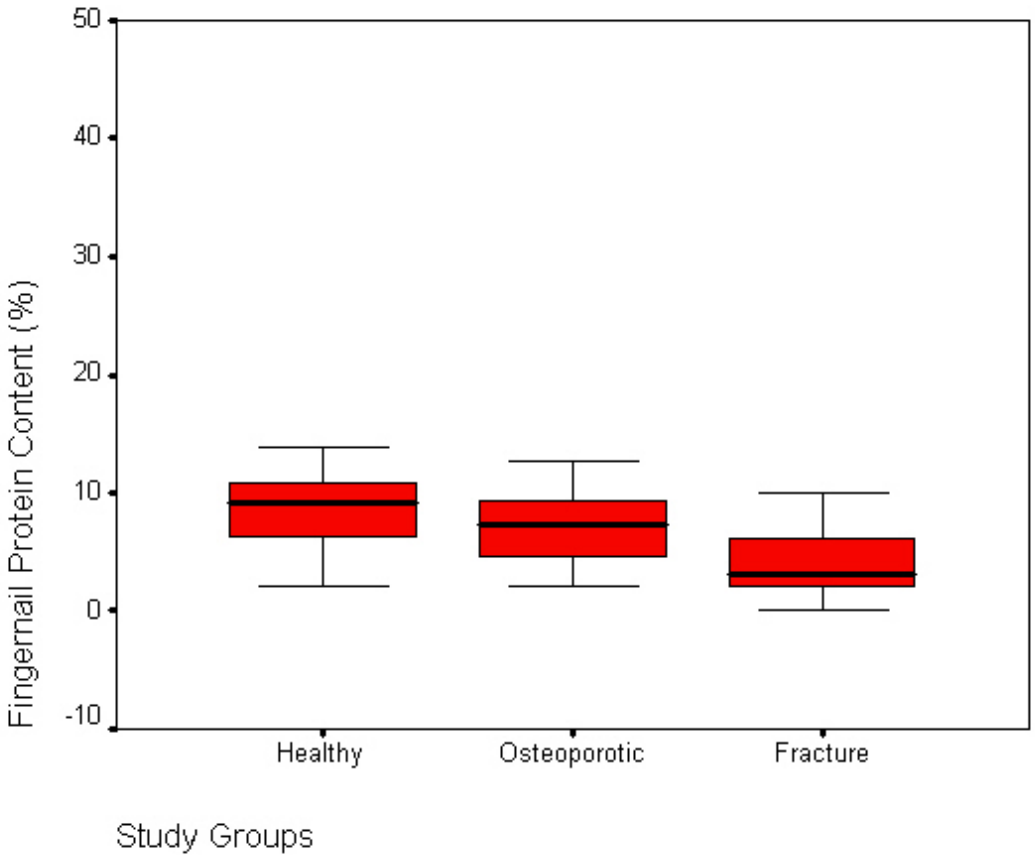

Fig. 1: Comparison fingernail protein content in three study groups

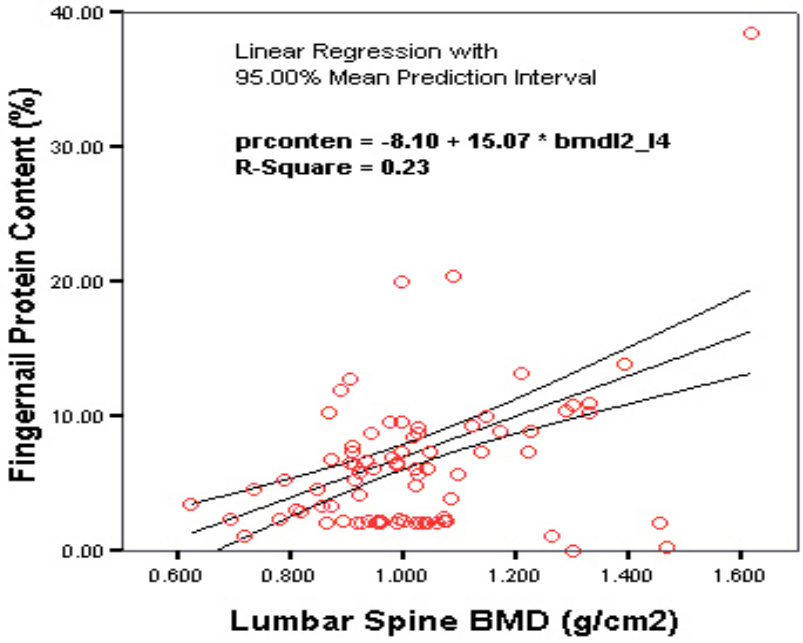

Fig. 2: Relationship between Fingernail Protein Content and Lumbar Spine BMD

Discussion

Prevention of bone fractures is critical to reduce osteoporosis related health costs and to improve quality of life of patients. There are several limitations in current way to predict fractures due to osteoporosis.

BMD is only one of the components play part to fractures risk augmentation; about 85% of the contribution to the fracture risk is independent on BMD, in general or age-related rise in fracture risk (4, 16).

In addition bone markers with or without anthropometric measures, offered little practical information for bone mass prediction (33). Thus bone quality measurement needs to new way and challenging in better estimation of this quality still continues.

The strength of bone is determined by its material composition and structure (10). Osteoporosis affects both the matrix and mineral components of bone resulting in a diminished resistance to fracture. Patients can suffer osteoporotic fractures despite normal bone mineral density. It can attributed to unmeasured influences of osteoporosis on protein phase of bone and its adverse effect interaction with depleted mineral phase (28). Bone collagen and nail keratin are two distinct structural proteins (18). Collagen synthesis, secretion, and deposition are equivalent with and harmonized with the other matrix proteins synthesis (27). Our results indicated that protein content of finger nail in osteoporotic patients with vertebral fracture is significantly lower than healthy women. In other study Raman spectroscopy was performed to assess the disulfide bond content of nail in two groups of patients, with and without osteoporosis at either the hip or lumbosacral spine (27). The spectroscopy data showed that the disulfide bond content of the nails obtained from osteoporotic patients was lower than that from healthy patients (27, 28). Also in similar study, the nail samples from 169 women, of which 39 had a history of osteoporotic fracture, were examined (34). Their results indicated that disulphide content of the nail reduced with age and was slightly higher in pre-, compared to post-menopausal women (P= 0.187). Significantly lower disulphide content was observed in nails obtained

from subjects with a history of fracture. When either disulphide content or BMD measured by DXA at the spine was used as a predictor, the odds ratios of these two measures were found to be comparable predictors for fracture status.

The relationship between nail and bone may exist in a measurable way. In the some experience suggested using of fingernails as a means of assessing bone health and was performed nano indentation to assess the degree of nail brittleness and Raman spectroscopy to assess the disulfide bond content of nail (28). These reports indicated that changes in the organic phase of bone are reflected in similar proteins, such as keratin, from which fingernails are composed.

Our study demonstrated that fingernail protein independently of age, BMI and BMD may predict vertebral fractures. Also our results show that fingernail protein content correlated with lumbar spine BMD and serum cross laps. This finding indicated that common pathways may involve in structural protein synthesis. Thus evaluation of extra skeletal accessories, such as hair, nail, and skin, may provide an estimation of bone quality, which would lead to a more complete evaluation of bone health. The fingernail protein may prove to be a valuable tool for assessing bone health.

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