

Research Article

Reciprocal Relation of Fetuin-A and Beta Crosslaps with Bone Health

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Abstract

Background

Over 90% of the Pakistani population being vitamin D deficient contributes tremendously to annually increasing trend of osteoporosis worldwide. Fetuin-A is one of the bone turnover markers which play a dynamic role in improving bone health. Similarly, serum beta Crosslaps (CTX) is a sensitive marker of bone resorption. This study aimed to correlate serum levels of fetuin-A and CTx with bone health in healthy females.

Methods

Total of 115 females of ages between 20 to 60 years were recruited in this cross-sectional study from Jinnah Postgraduate Medical Centre, Karachi. They were grouped as A & B on the basis of Bone Mass Density (BMD) T score > -1 and < -1, respectively. Anthropometric measurements were recorded and BMD was calculated by ultrasound bone densitometer (considering T-score ≥ -1 as normal). Serum was analyzed for bone minerals, vitamin D, CTX and fetuin-A. Data was analyzed statistically by SPSS 21, Mann-Whitney U test and Spearman's correlation (r) were applied where p value < 0.05 was considered significant.

Results

The complete cohort showed normal calcium levels while a low level of vitamin D was observed ($P > 0.05$). Interestingly, both serum fetuin-A and CTx levels were found high in group B as compared to group A ($p < 0.001$). Serum fetuin ($r = -0.718$, $p < 0.001$) and CTx ($r = -0.756$, $p < 0.001$) depicted negative correlation with BMD % and BMI, while their levels were positively associated to each other ($r = 0.481$, $p < 0.001$).

Discussion

Low values of BMD T-score (less than -1) are associated with high levels of fetuin-A in our female population. Raised fetuin-A in

the presence of low vitamin D and high CTx, suggests that serum fetuin-A does not reflect increased bone turnover. Further experiments are required to validate the role of fetuin-A in bone mineralization.

Keywords: Fetuin-A; Bone health; Bone mass density; CTx

Introduction

Bone health is mainly determined by a balance between bone formation and bone resorption, essential processes carried out by specialized cells. It is known that osteoclasts mediate bone resorption while osteoblasts form new bone [1]. An imbalance of this mechanism can lead to bone health problems like osteoporosis, which is an important issue faced by people all over the world.

Osteoporosis is known to cause over 8.9 million fractures annually [2]. Pakistan also faces increasing rates of osteoporosis, with currently 9.91 million people suffering from the disease; the number is predicted to increase to 11.3 million by 2020 and 12.91 million by 2050. Moreover, population of Pakistan is also challenged with an endemic of Vitamin D Deficiency (VDD). Conferring to a couple of studies conducted in Karachi, prevalence of VDD is 70-97% in healthy asymptomatic individuals [3].

Fetuin-A, also known as Alpha 2-Heremans-Schmid (AHSG) Glycoprotein is a non-collagenous protein that is stored in mineralized bone and teeth. It is derived from the hepatocytes and acts as a strong inhibitor of ectopic mineralization [4,5]. It is cleaved from a single chain precursor and its mature form contains two polypeptide chains [6]. Fetuin-A contains a cystatin-like protein domain and is responsible for inhibiting calpain, papain, cathepsin, and caspases by acting on cysteine peptidases. This enables fetuin-A to be a role player in a number of physiological and pathological processes. Most importantly cystatin domain-1 has a high affinity for calcium-rich minerals. Studies reveal that fetuin-A binds most strongly to mineralized bone by binding calcium and phosphorus together to form complexes [7]. Hence, it is a potent inhibitor of metastatic calcification [8]. It has also been hypothesized that fetuin-A works by promoting calcification within bone and inhibiting calcium precipitation within serum [9]. Moreover, remineralization of demineralized bone has also been proven to be possible within serum containing fetuin-A, and not with the one deficient of it, leading to a conclusion that fetuin-A has a direct effect on promoting bone mineralization [7].

The degradation products of collagen type I reflect the activity of osteoclasts, and can be assessed by measurement of telopeptides (CTX and NTx) and hydroxyproline or matrix proteins such as bone sialoprotein [10,11]. The most abundant form of collagen in the bone is Type I collagen which renders serum CTx (Carboxy-terminal Telopeptide) to be the most sensitive and specific test for bone resorption [10]. CTx is derived from type I collagen, which is the only type that is affected by osteoclasts and hence a true estimate of bone resorption can be obtained from this [12].

We hypothesize a link between fetuin-A, CTx and bone health. Our study aims to investigate bone health of healthy females, by

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employing serum levels of fetuin-A and CTx and their relationship with BMD percentage.

Material and Methods

Total of 115 females of ages between 20 to 60 years were recruited by convenient sampling method in this cross-sectional study after being approved by ethical review committee, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. An informed written consent was signed by each participant. Females on glucocorticoids, antiresorptive therapies, supplements, hormone replacement therapy or with any chronic diseases such as diabetes, coronary artery disease, liver disease and cancer were excluded from our study.

Data about the subjects' name, age, weight, height, physical activity, exposure to sun, and veil status were assembled by the primary investigator in a predesigned questionnaire. Anthropometric measurements were noted in standing posture and with light clothes, using Stadiometer (ZT-120 Health Scale, made in China). BMD of calcaneus (heel) bone (being a portable tool) was measured by ultrasound bone densitometer (Osteosys Sonost 3000 Bone Densitometry) where T-score ≥ -1 was considered normal). Grouping was done as group A & B on the basis of bone mass density T score > -1 and < -1 , respectively. All patients with BMD < 1 (either osteoporotic or at risk of osteoporotic) were recruited in group B.

About four ml of blood was collected in sterile venoject tubes. Serum was separated after centrifuging them at 2000xg for 5 minutes and then was aliquoted in small volumes and stored at -80°C until further use. The frozen serum samples were thawed right before analysis of calcium, alkaline phosphatase, vitamin D, CTx and fetuin-A. Calcium and alkaline phosphatase were assessed by enzymatic colorimetric kits available by Roche. Serum fetuin was analyzed by Enzyme Linked Immuno-Sorbent Assay kit method (Cat No.RD191037100 provided by BioVendor), while CTx was performed by the Elecsys 2010 analyzer (Roche Diagnostics). Vitamin D was estimated by commercially available ELISA kits (kit cat # KAP197; kit cat # KAP2281 by DIA source Immunoassays S.A. Belgium respectively). The following reference range for vitamin D levels were considered; deficient < 10 ng/ml, insufficient = $10 - 29$ ng/ml, sufficient = $30 - 100$ ng/ml and toxic = >100 ng/ml [13].

Data of continuous variables i.e. biophysical (age, height, weight, BMI, waist circumference, hip circumference, waist hip ratio, body fat, BMD) and biochemical parameters (calcium, alkaline phosphatase, vitamin D, CTx and fetuin-A) were statistically analyzed by employing SPSS (version 21; SPSS Inc., Chicago, IL, USA) and calculated as mean \pm Standard Deviation (SD). Comparison of means was done by utilizing Mann-Whitney U test. Correlation between serum CTx, fetuin-A and BMD percentage was established by Spearman's coefficient of correlation (r). The p-values < 0.05 were considered significant.

Results

A total of 105 age matched female subjects (mean age of 39.55 ± 6.91 years) were selected for this cross-sectional study. The results were achieved on the basis of body mineral density T-scores of the study subjects. The biochemical and biophysical considerations of the subjects are charted in table 1.

Variables	Group A (BMD T score ≥ -1) Mean \pm SD (n=56)	Group B (BMD T score < -1) Mean \pm SD (n=59)	P value
Age (years)	38.6 \pm 6.28	40.47 \pm 7.53	> 0.05
Weight (Kg)	72.6 \pm 16.23	67.10 \pm 12.32	> 0.05
Height (m)	1.5 \pm 0.03	1.6 \pm 0.04	> 0.05
BMI (Kg/m ²)	28.9 \pm 5.88	26.14 \pm 4.5	0.021
Calcium (mg/dL)	8.8964 \pm 0.55	8.84 \pm 0.48	> 0.05
Alkaline PO ₄ (IU/L)	205.32 \pm 76.57	216.20 \pm 93.57	> 0.05
Vitamin D (ng/mL)	16.22 \pm 9.41	16.90 \pm 12.86	> 0.05
Bone Mass Density (%)	90.86 \pm 8.9	67.46 \pm 7.78	< 0.001
Fetuin-A (mg/L)	45.15 \pm 12.39	70.97 \pm 10.49	< 0.001
CTX (ng/mL)	0.19 \pm 0.10	0.49 \pm 0.15	< 0.001

Table 1: Biophysical and Biochemical variables of both the groups.

The complete cohort showed normal calcium levels (mean 8.86 ± 0.51 mg/dL) thus no substantial difference was observed between the groups. With low levels of serum vitamin D (mean 16.56 ± 11.13 ng/ml), alkaline phosphatase (mean 210.5 ± 85.07 IU/L) was seen to be very high in all study subjects. BMI was found to be decreased in group B ($p = 0.021$). The serum CTx levels were detected high in group B as compared to group A ($p < 0.001$) but interestingly serum fetuin A levels were also revealed to be raised in individuals with low BMD T-score ($p < 0.001$).

Fetuin A was represented as negatively correlated with BMD percentage ($r = -0.718$, $p < 0.001$) and BMI ($r = -0.348$, $p < 0.001$). Furthermore, CTx ($r = -0.756$, $p < 0.001$) depicted negative correlation with BMD percentage while at the same time its levels were positively associated to fetuin-A ($r = 0.481$, $p < 0.001$) (Figures 1a - 1d).

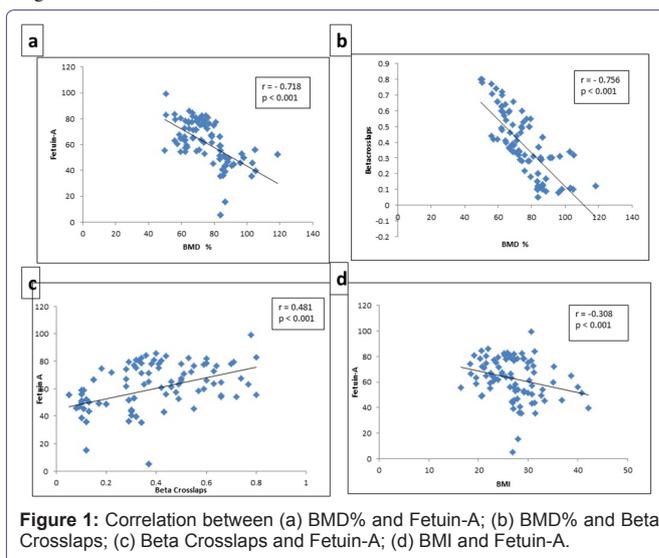


Figure 1: Correlation between (a) BMD% and Fetuin-A; (b) BMD% and Beta Crosslaps; (c) Beta Crosslaps and Fetuin-A; (d) BMI and Fetuin-A.

Discussion

Studies on relation of fetuin-A and CTx with BMD percentage are limited particularly in the Pakistani population which is transparently vitamin D deficient though does not exhibit any noticeable signs of declined bone health. This provides an area to investigate the link

between bone health markers (vitamin D, fetuin-A, CTx) and Bone health (BMD). To establish this link, we estimated the levels of vitamin D, fetuin-A, CTx and BMD and studied their correlation. The present study revealed high serum levels of alkaline phosphatase, CTx and quite interestingly of fetuin-A of individuals with low BMD percentage. However, calcium levels were indifferent in the overall study cohort (Table 1). In this part of the world, considering the cultural and ethnic backgrounds, comparative low levels of vitamin D are now considered as normal, as depicted by Arya et al., [14] and Roy et al., [15] in their studies. This generalized hypovitaminosis D could be a result of the genetic makeup of this population [16]. Further detailed genetic studies might be able to provide a causal relationship.

Increase in BMI directs towards presence of more adipose tissue in the body. Increase adiposity leads to the inflammatory condition in the body. Fat accumulation was observed to over-express fetuin-A mRNA in the liver of rats after inducing obesity; either by causing tissue insensitivity to insulin, by increasing fat metabolism or by limiting glucose clearance [17]. Furthermore, Stefan et al., indicated in a human study that fatty liver is associated with high plasma fetuin-A levels [18], this has been further proven by other studies [19]. However, Lavebratt et al., for the first time reported negative association between fetuin-A levels and fatness; this is consistent with our findings [20]. Study utilizing mediation analysis, suggested evidence of a causal relationship between the AHSR gene and BMD through fetuin-A and BMI mediators [21]. However, further genetic studies should be conducted to validate this fact in our population.

According to WHO criteria, individuals with BMD T-scores < -2.5 are osteoporotic and the ones between < -2.5 and -1 are at a high risk of developing osteoporosis, while T-scores > -1 are considered to be normal [2]. Our study depicts increased bone resorption in osteopenic subjects as suggested by the low BMD and high CTx levels [22]. CTx being an authentic bone resorption marker suggests that the bone is having osteoclastic activity going on for the release of minerals like calcium in the blood [23]. Previous studies have also endorsed CTx to be raised when the BMD decreases establishing it a reliable marker for bone health [24].

Another interesting finding is that of fetuin-A being high in individuals with decreased BMD percentage. According to a study conducted on elderly females, fetuin-A levels are associated with bone mass and bone resorption. Fetuin-A is a multi-functionality protein and leads to govern a number of mechanisms in the body by cytokine activation. It might have an effect on osteoclastic activity of the bone as it is strongly associated with CTx [25]. However, raised levels of fetuin-A have been associated with higher BMD suggesting fetuin-A to be promoting bone mineralization in elderly women [7].

The biphasic response of fetuin-A i.e., its ability to promote or inhibit calcification in different conditions has thus become a focus for researchers for its use as a potential bone marker of various bone disorders diagnostics [9]. However, regulation of fetuin-A is not very well explored. As both the markers are responsible for bone resorption, we further need to investigate that which of the two proteins is responsible for decreasing bone health in our population or both are working in synergism. Small sample size and evaluation of an inflammatory marker stands as a limitation of our study.

Physical activity acts as a powerful rehabilitation tool for osteoporotic patients. Vibration exercises strengthen the muscles and bones against the vibrating platform. Whole body vibration intensifies the level of hormones, averting osteoporosis. Exercises

based on piezoelectric theory, produce pressure induced bone formation by the variation of electrical potential, thus acting as a stimulant of the process of bone formation [26].

Conclusion

Bone resorption is high with high circulating fetuin-A and CTx in apparently healthy females which renders them at a higher risk for development of osteoporosis. The need to perform large scale studies in order to explore the interplay is warranted and is likely to give a better understanding of the regulation fetuin-A. Physical activity in terms of various trainings should be made a part of the individuals after a certain age.

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