

# Investigating Age Dependent Diversification of Bone Biomarkers in Females

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## ABSTRACT

**Background:** Bone is responsible to perform vital functions to provide support and maintain the structure of the body. Due to certain reasons, the bone encounters certain disorders which affect the bone functionality, especially aging in females.

**Objectives:** The study was designed to establish a relationship between biomarkers of bone metabolism with age & to analyze the occurrence of bone disorders with increasing age, specifically in females.

**Methodology:** Random samples of females were collected from the population of Karachi spliced as control with less than 30 years of age and as tests with late 30 years of age. The analysis was done exclusively with the detection of biochemical markers of bone turnover, including calcium, phosphorus, alkaline phosphatase, rheumatoid factor (RF) and C-reactive protein (CRP). The relationship between bone health, lipid profile, glycemic index and blood profile was also analyzed.

**Results:** Results depicted that alterations in normal serum concentrations of all biomarkers were frequent in elder females as compared to the younger ones.

**Conclusion:** In conclusion, the biomarkers of bone metabolism are closely related to age, evaluating that older females are more prone to the risk of developing bone diseases. The variables portrayed were useful in understanding their role concerning age in the development of bone.

### Keywords

Bone, biomarkers, Calcium, Rheumatoid Factor (RF), CRP, Blood profile

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## INTRODUCTION

Bone is a living, hard and metabolically active connective tissue that builds up the vertebral skeleton. Bone is mainly composed of minerals, organic matrix, water and lipids in decreasing proportions. Hydroxyapatite  $[Ca_{10}(PO_4)_6(OH)_2]$  a key component of bone is insoluble salt of calcium and phosphorus<sup>1</sup>. The principal function of bone is to provide mechanical support to the body, protection to the vital organs, maintaining structure, acid-base balance, mineral homeostasis and hematopoiesis. Several clinical

conditions are associated with bones including osteoporosis, fractures, tumors and bone cancer.

The process of bone formation is termed *osteogenesis* that happens on soft tissues during developmental stages or the healing processes. Bone modeling is the formation of bones on the existing bone tissues for proper morphological development, involving the changes in shape, curvature or thickness of bone. Bone remodeling is the coupled process of bone resorption followed by ossification on specific sites in the adult skeleton for the

maintenance of bone turnover and bone mass. The events of bone formation and resorption are strictly regulated by cytokines, growth factors and different systemic hormones<sup>2</sup>.

### Bone Biomarkers

Biomarkers are the quality features whose quantification and evaluation elucidate the altered physiological conditions. As indicated by the name, “biological markers” are the reflection of biological processes in an individual. Bone disease analysis is done exclusively with the detection of biological markers of bone along with imaging techniques and radiographic analysis. Some important bone biomarkers are:

- Biochemical constituents and enzymes as bone biomarkers including calcium, phosphorus, magnesium, alkaline phosphatase (ALP), calcitonin, osteocalcin, parathyroid hormone, vitamin D, C-reactive protein (CRP), rheumatoid factor (RF), type 1 collagen etc.
- Hematological indices as a bone marker
- Glycemic index as a bone marker
- Lipid profile as a bone marker

### Biochemical Constituents and Enzymes as Bone Biomarkers

Among biochemical constituents and enzymes as bone biomarkers, *calcium* is very important as it is the most abundant mineral in the body, of which 99% is stored in bones and teeth and the rest in other parts of the body. Its normal range and requirement depending upon age and gender. As in elderly people especially women, the risk of osteoporosis increases with a decrease in body calcium. The dietary requirement for an adult in a day range from 1000-1200mg. Moreover, *phosphorus* is the second most plentiful mineral of the body. It is among the main bone component i.e. Hydroxyapatite making bone strong. Its daily requirement for an adult is 700mg. Alteration in amounts of phosphorus in the body can lead to health conditions like joint pain and muscle fatigue. Excessive intake may also ruin bone health. *magnesium* being the third important bone biomarker is a necessary mineral that maintains the functioning of the heart, bones and blood pressure. Its recommended dietary allowance for adult women is 320mg and plays a vital role in maintaining bone health by coordinating calcium absorption and metabolism.

*Alkaline phosphatase (ALP)* is found in many tissues of the body including the liver, bone, intestine, and placenta. Its altered level in serum is normally indicative of two main courses of diseases: either hepatobiliary obstruction or bone disorder usually caused by an increase in osteoblastic activity.

*Calcitonin* is a hormone synthesized mainly in thyroid glands along with a variety of other tissues. Its reported main function is the involvement in phosphorus and calcium metabolism. It particularly drops calcium levels in the blood by acting upon parathyroid hormone and decreasing bone resorption. Besides calcitonin, another important mediator *osteocalcin* is also synthesized by osteoblasts exclusively plays a key role in bone mineralization as it contains Gla proteins, whose geometry completely fits the calcium structure to be incorporated in hydroxyapatite. However, the functions of osteocalcin associated with bone mineralization remain controversial<sup>3</sup>.

*Parathyroid Hormone (PTH)* is released from parathyroid glands and is known to increase blood calcium level. Its release is triggered by the magnesium levels in the blood. It increases the calcium concentration in the blood by either activating osteoclasts, reabsorbing calcium excreting from kidneys or enhancing vitamin D synthesis. PTH has also been reported for having anabolic treatment opportunities in bone diseases as in the case of osteoporosis<sup>4</sup>.

*Vitamin D* is a fat-soluble vitamin (also considered a hormone), sometimes plays an important role in calcium metabolism and homeostasis. The normal serum concentrations of 25-(OH)-Vitamin D is  $\leq 10\text{ng/ml}$ , contributing to bone diseases. Its deficiency can cause hyperparathyroidism, increased bone turnover, bone loss, and in extreme cases can also lead to osteomalacia<sup>5</sup>.

*C-reactive protein (CRP)* is an inflammatory marker usually associated with diseases like diabetes, cardiovascular diseases, lupus and rheumatoid arthritis. It is synthesized in the liver and increases in response to the extent of inflammation in the body. High sensitivity C-Reactive Protein (hs-CRP) is allied with a low mineral density of bones. *Another inflammatory biomarker is the Rheumatoid factor*, an autoantibody against IgG in humans usually detected for the diagnosis of rheumatoid arthritis i.e. an autoimmune disorder characterized by chronic

inflammation of joints. RF can also be checked in people with other autoimmune disorders<sup>6</sup>.

*Type 1 collagen* is chiefly found in bones along with soft tissues providing a matrix for mineralization. Procollagen Type 1 C-terminal peptide (PCIP) is secreted into the circulation by osteoblasts during early proliferative stages of bone formation which is followed by bone resorption being identified by the presence of cross-linked telopeptide of type 1 collagen<sup>7</sup>.

### Hematological Indices as Bone Marker

Hematological indices are also considered as a bone marker. Some studies demonstrated the positive association between bone health and blood profile regarding the influence of hematopoiesis and reported it to be a presumed indicator of bone mineral density (BMD)<sup>8</sup>. Studies further represent that in rheumatoid arthritis the haemoglobin and erythrocyte count decreases that stimulate the risk of anaemia, whereas no considerable change was observed in total WBCs. In another study, neutrophils-lymphocyte and platelet-lymphocyte ratios were reported as biomarkers of rheumatoid arthritis<sup>9</sup>.

### Glycemic Index as Bone Marker

Glycemic index is also considered as a bone marker e.g.: in diabetes type 1, the insulin deficiency results in altered bone metabolism because insulin has anabolic effects on bone metabolism that can preserve bone quality<sup>10</sup>. However, in type 2 diabetes, the body does not respond to the insulin produced with reduced risk of fractures and higher bone mineral density (BMD). Anti-diabetic medicines have also been found associated with decreased bone quality<sup>11</sup>.

### Lipid Profile as Bone Marker

While considering the lipid profile as a bone marker, recent studies based on epidemiological characteristics and clinical trials reported that high-fat mass contributes significantly to bone fragility and an extent of osteoporosis. Increased percentage of body fat is significantly associated with the increased risk of bone diseases, particularly osteoporosis, osteopenia, and non-spine fractures. Total cholesterol and low-density lipoprotein cholesterol had a negative correlation with bone mass density at the femur and spine. Decreased HDL levels can affect bone turnover as osteoblastic functions are disturbed due to the

inflammatory environment resulting in ultimately decreased bone mass<sup>12</sup>.

Other bone health predictors are obesity, BMI, and physical inactivity in which obesity can cause deleterious effects on bone as it can lead to fat deposition in bone marrow tissues. Several factors like ageing, diabetes, metabolic syndrome, and hormone impairment may lead to bone loss which could be overcome by maintaining a balance between diet and exercise<sup>13</sup>. Women with high abdominal fat have been reported to have the worst bone quality. Adipose tissues are associated with the secretion of certain hormones like estrogens in postmenopausal females and other adipokines which interferes with bone formation and resorption. Bone formation is enhanced as the result of increased weight and mechanical load although this can be inverted as lipotoxic effects of adipocytes<sup>14</sup>. BMI is considered a more approachable index when correlated with conditions like diabetes and hypertension. Sharmin *et al.* reported that bone loss was associated significantly with low BMI and ageing process<sup>15</sup>.

Although age, weight, and BMI reflect BMD, bone loss can also be influenced by other factors such as lifestyle and nutritional habits<sup>15</sup>. Physical exercises that focus on bone density are shown to enhance bone strength and cause a decline in bone loss and bone pain. The preservation of BMD in pre and postmenopausal women was reported in response to physical activity. For young adults despite sex variation, BMD is directly associated with physical exercise<sup>16</sup>.

Therefore; the current study was designed to establish a relationship between biomarkers of bone metabolism with age; to analyze the occurrence of bone disorders with increasing age specifically in females.

## MATERIALS AND METHODS

Healthy young female participants were included in the study. Blood samples of women were collected randomly from the population in Karachi. There was no specification considered rather than gender. In total, 55 samples were collected out of which 30 were considered as control and 25 were considered as a test, depending upon age i.e. females with <30 years of age were selected as control whereas females with >30 years of age were included in tests. Women who had amenorrhea due to hysterectomy or any other known causes like those taking any hormone

replacement therapy or on any bone-related medications, those with a history of thyroid disorders, jaundice and liver diseases were excluded from the study. A questionnaire regarding anthropometry was conducted along with all controls and tests models. Data including age, height, weight, habits, lifestyle and medical history was collected. Yellow capped clot activating gel vacutainer was used to collect serum; whereas purple capped ethylenediaminetetraacetic acid, (EDTA) vacutainer was used for plasma. Collected samples were centrifuged to separate serum /plasma which was then stored at 2-8°C. All samples were thawed before tests execution.

Parameters estimated calorimetrically including fasting blood glucose by following glucose oxidase method (Randox-Glucose-GLUC-PAP)<sup>17</sup>, cholesterol by cholesterol oxidase/peroxidase method (BioSystems)<sup>18</sup>, triglyceride by using glycerol phosphate oxidase/peroxidase method (BioSystem)<sup>19</sup>, HDL via phosphotungstate/mg-cholesterol oxidase/peroxidase method (BioSystems)<sup>20</sup>, LDL cholesterol was calculated using Friedewalds formula (Estimation of the concentration of low-density lipoprotein cholesterol in plasma) without using the preparative ultracentrifuge<sup>21</sup>. Calcium was estimated by Calcium MonoR by DiaCon Systems<sup>22,23</sup>. Enzyme activity of alkaline phosphatase was estimated via the kinetic method by using p-Nitrophenylphosphate as substrate (LAB KIT)<sup>24,25</sup>. Phosphorus-phosphomolybdate (Spinreact) was used for the estimation of phosphorus on UV-Visible Spectrophotometer (Jenway 6305)<sup>26</sup>. The CRP level was determined by a quantitative turbidimetric method using Atlas Medical-CRP Latex kit<sup>27,28</sup> while, rheumatoid factor (RF) was determined by Atlas Medical – RF Latex kit<sup>29,30</sup>.

## RESULTS

**Table 1. Statistical Results of Anthropometric Data.**

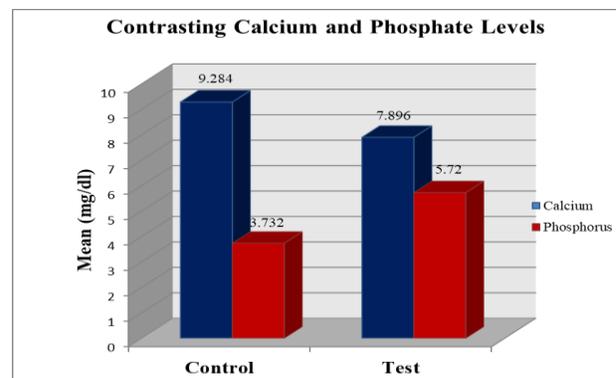
S. No.	Parameter	Age	Height	Weight	BMI
01	Control (n=30)	23.35 ± 0.27	52.37 ± 1.58	158.044 ± 1.980	21.140 ± 0.733
02	Test (n=25)	58.13 ± 2.41	66.67 ± 1.58	162.983 ± 2.014	25.190 ± 0.682

*n* = No of subjects..

Student *t*-test was done to calculate the *p*-value, the significance level was assumed to be < 0.05.

Values are represented as MEAN ± SEM.

Total 55 females (30 control & 25 test subjects) fulfilling the selection criteria were allocated. The mean age of control is 23.35 ± 0.27 years and 58.13 ± 2.41 years for test subjects anthropometric data are given in Table 1. The biochemical profiling of this study included the evaluation of fasting glucose levels, HbA1c, lipid profiling, alkaline phosphatase activity, calcium and phosphorus serum levels as well as analysis of CRP and RA factor in the subjects. The results of biochemical parameters including enzymatic assay and determination of CRP and RA were compared in test and control subjects. No significant difference in FBG and HbA1c were found in a test as compared to the control group. Whereas a significant increase in lipid profile including cholesterol, triglycerides, LDL was found in test subjects while comparing with control (Table 2). The study demonstrated that calcium levels drop-off with the increase in age (*P* = 0.0227), whereas phosphorus levels rise with the increasing age (*P* < 0.0001); showing the inverse relationship between calcium and phosphate levels i.e. the decrease in serum concentration of calcium causes the increase in phosphorus concentration in serum and vice versa (Fig. 1).



**Figure 1.** Comparison of calcium and phosphorus levels in control and test subjects

**Table 2. Statistical Results of Quantitative Biochemical Parameters.**

S. No.	Parameters	Control (n = 30)	Test (n = 25)	P-Value
01	GLUCOSE (75-115mg/dL)*	91.349 ± 4.151	104.208 ± 7.574	P = 0.1149
02	HbA1c (4-5.6%)*	4.811 ± 0.140	5.266 ± 0.251	P = 0.0953
03	CHOLESTEROL (200-239mg/dL)*	185.217 ± 7.877	225.318 ± 10.729	P = 0.0036
04	TRIGLYCERIDES (150-199mg/dL)*	138.975 ± 9.332	191.253 ± 10.628	P = 0.0007
05	HDL (> 60mg/dL)*	67.956 ± 2.958	81.877 ± 7.583	P = 0.0581
06	LDL (130-160mg/dL)*	89.465 ± 10.035	105.189 ± 9.571	P = 0.2869
07	ALP (60-170U/L)*	42.33 ± 2.693	96.125 ± 3.395	P = 0.0001
08	CALCIUM (8.5-12.0mg/dL)*	9.284 ± 0.323	7.896 ± 0.532	P = 0.0227
09	PHOSPHORUS (2.5-5.0mg/dL)*	3.732 ± 0.298	5.720 ± 0.340	P < 0.0001
10	CRP (mg/dL) *(1-3mg/L)	1.85 ± 0.23	4.38 ± 0.25	P < 0.0001
11	RF	+ (less than 5%)	+++ (approx 80%)	

*n* = No of subjects.

Student *t*-test was done to calculate the *p*-value, the significance level was assumed to be < 0.05.

Values are represented as MEAN ± SEM.

Therefore, it has been evidenced that the increasing age has negative effects on bone biomarkers resulting in poor bone quality and requiring the increased aid of health assistance as the life proceeds. ALP and phosphorus levels were found to increase in test subjects while comparing with control. A significant decrease was found in HDL and calcium level in a test as compared with control subjects. CRP and RA factor analysis revealed that C-reactive protein was found to be significantly increased in test subjects as compared to the control group. While the RA found to be positive (approx 80%) only in test subjects in comparison to control subjects (less than 5%) indicating the presence of bone-related inflammation in these subjects.

## DISCUSSION

Biomarkers of bone metabolism are closely related to age. This study evaluated that elder female are more prone to the risk of developing bone diseases when compared to younger females. This indicates the hazard of bone deterioration with the growing age.

### Blood Glucose Level and Bone Health

Diabetes and osteoporosis are among the most prevalent diseases of the era. Altered levels of blood glucose levels are found to be associated with altered bone metabolism, which results in decreased bone quality. Diabetes type 1 is associated with insulin deficiency and results in altered bone metabolism because insulin has anabolic effects on bone metabolism that can preserve bone quality<sup>10</sup>. However, in diabetes type 2, the body does not respond to the insulin produced with reduced risk of fractures and higher BMD. This study demonstrated an increase in blood glucose levels and HbA1c scores in elder females when compared with the control group, illustrating that aged women are more prone to the risk of diabetes as well as bone disease.

### Lipid Profile as a Bone Marker

This study showed a significant increase in cholesterol and triglycerides levels in elderly women, exhibiting the risk of decreased bone quality. Previous studies also reported the risk of low BMD with increased cholesterol and triglycerides levels<sup>31</sup>. Another study reported the positive association between lipid profile and osteoporosis in postmenopausal

women however no significant relation between BMD and osteoporosis was reported<sup>32</sup>.

### Alkaline Phosphatase as a Bone Marker

High levels of ALP are usually associated with higher age regardless of the fact of presence of isozymes which can indicate several clinical conditions. Although in this study, no significant relationship between elderly and young females was observed, an increase in mean serum concentrations was observed for elder females. Another study reports a significant increase in ALP in postmenopausal women with an inverse relation to serum calcium levels<sup>33</sup>.

### Calcium as a Bone Marker

The calcium level of the test group was significantly decreased pointing to overage as the dominating reason for the risk of bone health disruption, as low calcium levels cause an increase in bone resorption<sup>34</sup>. The reason behind low calcium levels in elder people can be malabsorption, one study reported that calcium absorption decreases significantly as age increases especially after 60 years of age<sup>35</sup>. That could be related to the declined levels of estrogen and other hormones at menopause<sup>35</sup>.

### Phosphorus as a Bone Marker

In this study, phosphorus was significantly increased in the test group. Increased phosphorus has deleterious effects on bone and can also relate to heart disease. It has been reported that low blood pressure was associated with high phosphorus<sup>36</sup>. Hypophosphatemia is also associated with osteomalacia as it will cause the impaired formation of hydroxyapatite. A finding showed that long term exposure to high phosphorus can lead to bone impairment<sup>37</sup>.

### Association between Calcium and Phosphorus

This research depicts the inverse relation between calcium and phosphorus levels i.e. high phosphorus and low calcium in the test group and vice versa. A negative relation between skeletal health and low calcium and phosphorus ratio was reported. Thus, the risk of development of bone disease in older females was higher than the younger ones. Calcium and phosphorus are dependent on parathyroid hormone and vitamin D levels; increased phosphorus has disastrous effects on bone whereas increased calcium levels have protective effects<sup>38</sup>.

### C-Reactive Protein (CRP) as a Bone Biomarker

C-reactive protein is an inflammatory marker produced by the liver, indicating tissue damage. Bone health is closely associated with CRP, as high CRP levels indicate poor bone health and increased fracture risk<sup>39</sup>. Several studies do not report CRP as an indicator of low BMD, but relate it as the cause of bone loss, poorer bone quality, and increased fracture risk and joint destruction<sup>40</sup>.

### Rheumatoid Factor (RF) as a Bone Biomarker

Rheumatoid factor is an antibody targeted against persons own tissues, resulting in synovial inflammation, leading to rheumatoid arthritis. The solitary study showed 16.66% positive results for the test group that included elder females thus, indicating the risk of rheumatoid arthritis in aged women. Another study reported that Anti-Citrullinated Protein Antibodies (ACPA) were also associated with the risk of RA<sup>41</sup>.

### Treatment and Prevention of Bone Diseases

Antiresorptive drugs are effective in preventing bone fractures in combination with anabolic therapy. Specific treatments are also available for specific bone conditions. Bone diseases can be prevented by meeting the basic nutritional needs to maintain bone health. Avoiding alcohol and smoking can also be beneficial. An appropriate combination of physical activity and weight should also be managed in a routine to maintain bone health. Maintaining adequate exposure to sunlight and dairy intake can also aid in maintaining bone health. Elderly people should also manage to see doctor and routine checkup to reduce the risk of fractures. Awareness regarding health state can also be considered.

## CONCLUSION

In conclusion, the alteration of biochemical markers of bone in association to increasing age is intact enough to be considered as the strong predictor of bone health that regards older females at higher risk of developing bone diseases. The parameters analyzed, discussed and described in this study coordinated well in analyzing the role of age in the occurrence of bone disorders, particularly in females. Results exposed that elderly women are at risk to encounter bone disorder at the late ages of their life. Therefore, women should maintain compatibility among their dietary habits and exercise routine to avoid ending up

in a diseased state. Medical assistance should be taken regularly to monitor bone health and maintaining bone quality.

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## LIST OF ABBREVIATIONS

ACPA	Anti-Citrullinated Protein Antibodies
ALP	Alkaline Phosphatase
BMI	Body Mass Index
BMD	Bone Mineral Density
EDTA	Ethylenediaminetetraacetic acid
FBG	Fasting Blood Glucose
HDL	High-density Lipoprotein
hs-CRP	High sensitivity C-reactive protein
IgG	Immunoglobulin-G
PTH	Parathyroid hormone
PCIP	Procollagen Type 1 C-terminal peptide
RA	Rheumatoid Arthritis
RF	Rheumatoid factor

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