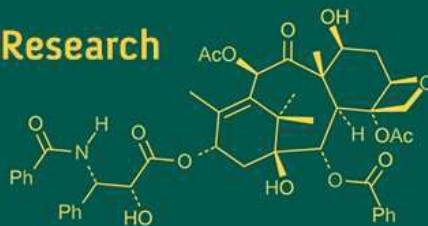
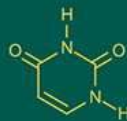


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(9): 634-636
www.biochemjournal.com
Received: 19-06-2024
Accepted: 22-07-2024

Dr. Suman Medhi
Tezpur Medical College and
Hospital, Assam, India

Dr. Deepika Lahon
Tezpur Medical College and
Hospital, Assam, India

Dr. Chinmoy Das
Tezpur Medical College and
Hospital, Assam, India

Dr. Maireyee Bora
Tezpur Medical College and
Hospital, Assam, India

Study of osteogenic minerals in osteoporosis patients

Dr. Suman Medhi, Dr. Deepika Lahon, Dr. Chinmoy Das and Dr. Maireyee Bora

DOI: <https://doi.org/10.33545/26174693.2024.v8.i9Sh.2187>

Abstract

Background: Osteoporosis is a condition characterized by a reduction in bone mineral density and bone mass, or alterations in the structure and strength of bones. These changes can result in weakened bones, increasing the likelihood of fractures. The aim is to estimate the levels of serum calcium, phosphorus and vitamin D in osteoporotic patients.

Methods: This observational study was conducted under the department of Biochemistry and Department of orthopedics at Tezpur Medical College and Hospital with 50 diagnosed cases of osteoporosis.

Results: The results were analysed using appropriate statistical test. The data compiled were analysed with GraphPad InStat® 3 Statistical Software. In our study we found out the osteogenic minerals represented by calcium and phosphorus and vitamin D levels were significantly high than the normal values.

Conclusion: From our study it can be concluded that the levels of serum calcium and vitamin D levels are reduced and serum phosphorus levels within the normal range in osteoporotic patients.

Keywords: Osteoporosis, calcium, phosphorus, vitamin D

Introduction

Osteoporosis is the most common metabolic bone disease in the world. It is the second leading cause of musculoskeletal morbidity in elderly. By definition it is an abnormal reduction in bone tissue mass per unit volume of anatomical bone ^[1], causing skeletal weakness, although the ratio of mineral to organic elements is unchanged. It is characterized by reduction in the thickness of the cortex specially from endosteal surfaces with the medullary contents being made up of a yellow and fatty cancellous bone tissue. There is reduction in the number and size of trabeculae and in the number of osteoblast present which leads to fracture or crushes with minor trauma ^[2].

Diagnosing osteoporosis involves a comprehensive approach that includes clinical evaluation, bone mineral density (BMD) testing, and laboratory assessments. The gold standard for diagnosing osteoporosis is dual-energy X-ray absorptiometry (DEXA or DXA), which measures BMD at the hip and spine. A T-score of -2.5 or lower confirms osteoporosis. Additionally, laboratory tests are conducted to measure serum calcium, vitamin D, phosphorus, and parathyroid hormone (PTH) levels to rule out secondary causes of bone loss. Markers of bone turnover, such as bone-specific alkaline phosphatase (BSAP), osteocalcin, C-terminal telopeptide (CTX), and N-terminal telopeptide (NTX), are also assessed to evaluate bone formation and resorption rates. Imaging techniques like vertebral fracture assessment (VFA) using DXA or spinal X-rays may be used to detect vertebral fractures, which are common in osteoporosis but often asymptomatic. This multi-faceted diagnostic approach ensures accurate identification and management of osteoporosis ^[3].

Calcium along with phosphate is required for the formation of hydroxyapatite and physical strength of skeletal tissue. Bones which are in a dynamic state serve as reservoir of calcium ^[4]. The hormone calcitriol (Vitamin D), parathyroid and calcitonin are the major factor that regulate plasma calcium within a normal range 9-11 mg/dl. Calcium is absorbed actively in the duodenum by an active carrier mediated process using a calcium binding protein. Above the age of 16 years where there is gradual decline in the intestinal absorption of calcium which may lead to senile osteoporosis ^[5].

Corresponding Author:
Dr. Suman Medhi
Tezpur Medical College and
Hospital, Assam, India

Calcium is the fifth most common element in the body and the most prevalent cation. Hypocalcemia may be due to a reduction in either the albumin-bound calcium or the free fraction of calcium, or both.

Phosphorus in the form of inorganic and organic phosphate is an important and widely distributed element in the human body. Hypophosphatemia, or phosphate depletion may be caused by a shift of phosphate from extracellular to intracellular spaces, renal phosphate wasting, decreased intestinal absorption, and loss from intracellular phosphate.⁵

Objective of the study

The aim and objective of this study is to estimate the levels of serum calcium, phosphorus and Vitamin D in osteoporotic patients.

Materials and Methods

This was an observational study, which was conducted under the Department of Biochemistry in association with Department of Orthopedics, Tezpur Medical College and Hospital, Tezpur after approval from the Hospital Ethics Committee and after obtaining patient's attendant's informed consent. The study was carried out in 50 patients who were diagnosed cases of osteoporosis.

Inclusion criteria

1. Diagnosed cases of osteoporosis.

Exclusion criteria

1. Cases other than osteoporosis like osteoarthritis, osteomalacia, osteopenia any musculoskeletal defect, trauma, any other pathogenic fracture.
2. Alcoholic patients, cirrhosis, pregnant woman, cancer patient, other autoimmune diseases, hepatitis, malaria, dengue.
3. Patients receiving treatment with calcium or phosphorus or vitamin D supplements
4. Patients not willing to participate in the study.

Cases taken in the study were amongst those who were admitted in the department of orthopaedics Tezpur medical college and hospital with different types of clinical manifestations of osteoporosis, the diagnosis of which were made on the basis of x-ray findings, clinical examinations and laboratory findings.

Methods of evaluations

Estimation of serum calcium, phosphorus and Vitamin D was done in vitro 5600 which works on the principle of spectrophotometry. The serum was separated and centrifuged for 3 min at 3000 rpm in a clinical centrifuge machine.

Sample Analysis

Estimation of Calcium on Vitros 5600

Principles of the Procedure

The Calcium test is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by spreading layer to the underlying layers. The bound calcium is dissociated from binding proteins, allowing the calcium to penetrate the spreading layer into the underlying reagent layer. There, the calcium forms a complex with Arsenazo III dye, causing a shift in the absorption maximum.

After incubation, the reflection density of the colored complex is measured spectrophotometrically. The amount of coloured complex formed is proportional to the calcium concentration in the sample

Estimation of Phosphorus on Vitros 5600

Principle and Procedure

The phosphorus test is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by spreading layer to the underlying layers. Inorganic phosphorus react with ammonium molybdate to form a phosphomolybdate complex.

Estimation of Vitamin D on Vitros 5600

Principle and Procedure

A competitive immunoassay technique is used which involves the release of the 25-OH Vitamin D in the sample from the binding protein using a low pH denaturant and the subsequent competition of the free 25-OH Vitamin D with horseradish peroxidase (HRP) labelled 25-OH Vitamin D reagent for monoclonal anti-Vitamin D bound to the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction.

Results

In our study there were 32 female subjects and 18 male subjects. The study subject's age varied within the range of 40-70 years with a mean age of 64.6 ± 1.52 and a median age of 60 years.

Age interval	Number of patients
40-50 years	12
50-60 years	20
60-70 years	18

Serum calcium

The values ranges from 5.8-7.4 mg/dL with mean value 6.14 ± 0.56 mg/dL and maximum number of subjects have serum calcium activity in the class interval of 5.0-6.0 mg/dL.

Serum phosphorus

The values ranges from 3.2-4.6 mg/dL with mean value 3.74 ± 0.44 mg/dL and maximum number of subjects have serum phosphorus activity in the class interval of 3.4-4.0 mg/dL.

Serum vitamin D

The values ranges from 10-20 ng/mL with mean value 14.0 ± 0.24 ng/mL and maximum number of subjects have serum phosphorus activity in the class interval of 10.0-12.5 ng/mL.

Discussion

Osteoporosis is a bone disease marked by a gradual loss of bone density and an elevated risk of fractures. This condition often remains symptom-free until fractures occur, which can happen even with minor injuries. In postmenopausal women over 50, these fractures account for up to 80% of all such injuries. The impact of osteoporosis is significant, posing a substantial medical burden on the elderly and placing a heavy economic strain on the healthcare system. However, early diagnosis can prevent osteoporosis-related fractures, and appropriate treatment can reduce the risk of additional fractures even after an initial one has happened.⁶ The diagnosis of osteoporosis involves a multifaceted approach, combining clinical assessment, bone mineral density (BMD) testing, and laboratory evaluations to assess bone health and exclude secondary causes of bone loss. The primary diagnostic tool is dual-energy X-ray absorptiometry (DEXA or DXA), which

measures BMD at critical sites like the hip and spine, with a T-score of -2.5 or lower indicating osteoporosis (Kanis *et al.*, 2019) ^[7]. Laboratory tests include measurements of serum calcium, vitamin D, phosphorus, parathyroid hormone (PTH), and markers of bone turnover such as bone-specific alkaline phosphatase (BSAP), osteocalcin, C-terminal telopeptide (CTX), and N-terminal telopeptide ^[8]. These tests help identify potential secondary causes like hyperparathyroidism or vitamin D deficiency. Additional imaging, such as vertebral fracture assessment (VFA) using DXA or spinal X-rays, may be conducted to detect vertebral fractures, which are often asymptomatic yet common in osteoporosis patients (Eastell *et al.*, 2016) ^[9]. This comprehensive diagnostic approach ensures accurate identification and effective management of osteoporosis.

This study was carried out in the Department of Biochemistry in association with Department of Orthopedics, Tezpur Medical College and Hospital where we evaluated the levels of osteogenic minerals serum calcium, phosphorus and vitamin D levels in diagnosed cases of osteoporosis. The study subject's age varied within the range of 40-70 years with a mean age of 64.6 ± 1.52 and a median age of 60 years with higher incidence of females (64%) compared to males (36%). Khadilkar *et al.* concluded that the incidence of osteoporosis, characterized by reduced bone mass and compromised bone structure, is rising among women in India. Although prevalence data come from various small-scale studies across the country, estimates indicate that out of the 230 million Indians over 50 years old in 2015, around 20%, or approximately 46 million, are women with osteoporosis. This makes osteoporosis a significant public health issue among Indian women. Factors such as low calcium intake, widespread vitamin D deficiency, increased life expectancy, gender inequality, early menopause, genetic predisposition, limited diagnostic facilities, and poor awareness of bone health contribute to the high prevalence of this condition which is a similar finding as of our study in terms of prevalence of osteoporosis in female population ^[10].

In our study we found out that serum calcium levels are reduced in osteoporotic patients and the values ranges from 5.8-7.4mg/dL with mean value 6.14 ± 0.56 mg/dL. Chen *et al.* ^[11] carried out a study and found that lower serum calcium levels were common among patients with osteoporosis which is a similar finding like our study. In our study we found out the serum phosphorus levels to be in the range of 3.2-4.6 mg/dL with mean value 3.74 ± 0.44 mg/dL which is a similar finding like the study done by Tomita A. ^[12]. The levels of vitamin D ranges from 10-20ng/mL with mean value 14.0 ± 0.24 ng/mL which is lower than the normal range as found by study done by Voulgaridou *et al.* ^[13].

Conclusion

From our study done on 50 patients of diagnosed osteoporosis it can be concluded that the levels of serum calcium, vitamin D levels are reduced whereas the levels of serum phosphorus remains in the normal range. These three biochemical markers can be utilized for the definitive diagnosis of osteoporosis along with bone mineral density (BMD) measurements.

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