

Protocol+for+determination+of +calcium+in+Osteoporosis+pati ent.docx

by PSKJDH GUWAHATI

Submission date: 17-Jul-2025 10:03AM (UTC-0500)

Submission ID: 2695012095

File name: Protocol_for_determination_of_calcium_in_Osteoporosis_patient.docx (122.68K)

Word count: 2773

Character count: 15044

Protocol for determination of calcium concentration in blood sample as a marker for Osteoporosis

Koni Potom, Dotu Gamlin, Vijay Kumar Tilak, Sanjay Rawat¹

¹ Faculty of Pharmaceutical Sciences, Apex Professional University, NH-515, Pasighat Smart City, Arunachal Pradesh-791102, India.

Abstract

Osteoporosis is a bone disease primarily occur in female. However, men are also affected by the disease. The disease is manifested through the number of factors including hormonal deregulation, deficiency of vitamins and abnormal metabolism. Calcium is one of the essential micronutrients required for the growth and development of bone in human body. The calcium level is elevated in osteoporosis. Therefore, growth of human body is dependent on the dietary intake of calcium. The hormones calcitriol and parathyroid hormone (PTH) control the level of calcium in human and other primates. The 99% of calcium in bone is stored as hydroxyapatite. Constant irregular level of calcium in blood causes the osteoporosis condition. In osteoporosis, density of mineral calcium has been found to be drastically decreased in bones. This in turn, decreases the bone mass. The increase concentration of calcium in blood due to bone desorption might lead to hypercalcemia. In blood circulation, calcium exists in three forms 1) free (ionized form), 2) complexed (chelated form) and 3) protein bound form. Therefore, measuring blood calcium level is important in diagnosing and preventing several diseases. The total calcium level and ionized calcium level are the two tests generally performed on blood sample. Herein, for the first time we are reporting the laboratory based qualitative and quantitative identification test for determination of blood calcium level for osteoporosis disease.

Key words- Blood calcium level, laboratory protocol, osteoporosis, calcium, chelation.

Corresponding author- Dr. Sanjay Rawat

Email- rawat@apexuniversity.edu.in

Introduction

Osteoporosis is a disease of bone that generally occur in older people. The decrease in mineral density and bone mass lead to decrease in bone strength. This might cause the increase probability of bone fracture. The additional changes in bone structure may lead to internal injuries and tissue damage. The older people and postmenopausal women easily develop osteoporosis and osteoarthritis (1). As per World Health Organization (WHO), the young and healthy women tend to develop osteoporosis if bone mineral density (BMD) is less than 2.5 (T score < 2.5). The two important cells involve in shaping the bone structure and density are osteoclast and osteoblast cells (2). The osteoclast and osteoblast cells are under the strong influence of hormones like testosterone in male and estrogen, progesterone in female. However, thyroid hormones, parathyroid hormone (PTH), calcitriol and calcitonin are the common hormones that too play a significant role in regulation of calcium level and thus the bone density (3). The bone is primarily composed of 99% hydroxyapatite. The discussed hormones regulate the calcium signaling and concentration in blood and bone cells. Increase in calcium level caused by calcitonin hormone induces the differentiation of osteoclast cells and its reabsorption into bone. However, osteoblast cells regulate the activity of osteoclast cells and calcium homeostasis. This in turn, lead to deposition of more matrix in the bone. The hormone calcitriol instead increases the blood calcium level. Therefore, more depletion of calcium from osteoclast and osteoblast bone cells increases blood calcium level (4). The parathyroid hormone (PTH), however, regulates the extracellular calcium level via vitamin D based metabolites. The osteoblast cells are regulated by PTH helps in mineralization of calcium and phosphate in the bone by increasing calcium transport. Thus, bone density and strength are affected by PTH. The more the level of calcium in bone, the more will be the attachment of osteoclast cells hence more dense and strong bone. Loss of bone mass due to decreased bone mineral density (BMD) is the primary cause of change in bone architecture. The architectural changes lead to fragile bone structure. Thus, the fragility of bone is directly proportional to the calcium deposition and reabsorption into the bone matrix. The skeletal tissue and bones are under constant influence of the hormones that regulate the activities of osteoblast, osteoclast and osteoid cells present in the bone matrix. Bone matrix itself composed of collagenous and non-collagenous proteins, that form a mesh for the mineral deposition in the form of hydroxyapatite. To increase the bone density the mineralization process takes place in the osteoid organic part of the bone matrix (5). In definite ratios the calcium and phosphorous ions are deposited in the form of hydroxyapatite. Thus, the toughness and biomechanics of the bone is the direct results of the availability of calcium ions and formation of hydroxyapatite (6). The process of bone remodeling and resorption is well under control of osteoclast and osteoblast cells. However, hormones estrogen, PTH, calcitonin and vitamin D regulate the mesenchymal stem cells (MSC) which form the osteoblast cells. The osteoclast cells control the differentiation and activity of osteoblast cells. The osteoblast cells in turn, are regulated by hormones PTH and calcitonin for increasing or decreasing the activities of osteoclast cells (7-8). Therefore, even the bone and bone cells are under the constant influence of hormones and vitamin D, the ultimate demand of calcium is either fulfilled by dietary intake of calcium or decreasing the bone matrix density in a person. The calcium supplements that might help in younger individuals to regain bone strength, However, in postmenopausal women and older individuals above 60 years of age calcium absorption is poor. Moreover, the osteoclast-osteoblast remodeling and resorption capacity of the bone is drastically decreases in old age individual. Hence, fragile and weak bone might easily get fractured. The condition of osteoporosis could lead to increase blood calcium level (9). Therefore, determining the blood calcium concentration could be an easy and early predictor of osteoporosis. Herein with this study, we are claiming a new chemical method development for the early screening of osteoporosis using blood samples from a patient. The method is cost effective, requires few chemicals and 15 minutes of a time to distinguish between a healthy person and a patient suffering from osteoporosis. The test could be categorized under normal test for hypercalcemia and bone health (10-11). Further development on the point of care paper based diagnostic device for identification of severity of osteoporosis is under development for postmenopausal women.

Methodology for determination of blood calcium level

- 1) Take out the blood sample from a patient using adequate sterile syringe and needle by making a small puncture in the visible vein of one arm (5 mL) (12-14).
- 2) Sample is collected in sterilized container.

- 3) Small amount of anticoagulant (Heparin) is added in to the blood in the container. The samples are kept at 4 °C in a refrigerator.
- 4) 6 test tubes are labeled as A, B, C, D, E & F.
 Test tube A- blood from healthy person.
 Test tube B- blood from osteoporosis patient.
 Test tube C- Test tube without any EDTA.
 Test tube D- Test tube without any blood.
 Test tube E- Test tube without any HCl.
 Test tube F- Test tube without any ammonia solution.
- 5) 1 mL blood was taken in each hot air oven sterilized test tubes. However, in test tube D, instead 1 mL distil water was added.
- 6) Prepare 0.1 molar (M) 100 mL solution of ethylene diamine tetra acetic acid (EDTA).
- 7) Except test tube C, 0.1 M 1 mL solution of EDTA was added to other labelled test tubes.
- 8) Each test tube mixed gently and heated for 1-2 minutes at 25 °C on a water bath.
- 9) Spin off each test tube for 30 minutes in a centrifuge.
- 10) Separate the supernatant and discard the pellet.
- 11) The supernatant contains the EDTA complexed with calcium in a chelate form
- 12) Except test tube C, 1 drop of concentrated hydrochloric acid (HCl) was added to other supernatant containing labelled test tubes.
- 13) The displaced Calcium from EDTA chelate, was qualitatively and quantitatively measured for free calcium.
- 14) Except test tube F, 1 drop of ammonia solution was added to each labeled test tube.
- 15) Compare the formation of deep blue-black color in each test tube

Figure 1

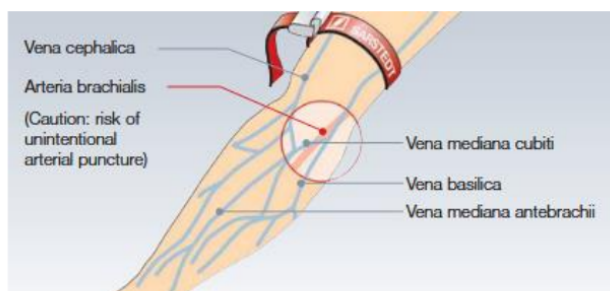


Fig.1 Point for vein puncture and blood sample collection from hand (Source- Labowarte von A-Z, 3rd edition, Kolhhammer Verlag, Stuttgart, Germany, 100 pages)

Results and discussion

Osteoporosis is a huge healthcare challenge in society. The outcomes of old age and hormonal imbalance could lead to severe bone health distortion. The primary inorganic elements that are required for maintaining bone structure and density are calcium and phosphate (15). The inadequate regulation and absorption of calcium element is the leading cause of bone related diseases like osteoporosis. In any bone the hydroxyapatite is the chemical compound that is formed out of these inorganic elements. The hydroxyapatite, in turn enables the formation of matrix and provides template in all three dimensions. This, in turn provides the density and structure to the bone. In the process of increasing bone density and strengthening bone structure both osteoblast and osteoclast cells play important roles in maintaining adequate calcium influx from the blood to bone. Therefore, any upregulation of calcium level in the blood could be marked as identification of decrease influx of calcium into the bone. Hence, the diseases of bone density like osteoporosis, could be easily identified by measuring the

rise in calcium concentration in the blood (16-18). Therefore, early diagnosis of osteoporosis could be achievable to identify the disease and decrease the female mortality.

Figure 2



Fig. 2 Set of six labelled test tubes

Early diagnosis with minimum and inexpensive tools is the demand of large population live in developing countries. Here, in this study a laboratory setup simple protocol has been design and formulated. The step of protocol is simple and inexpensive. It requires minimum chemical reagents and could be incorporated into pathological testing of osteoporosis. The blood test involves the withdrawing 3-5 mL of blood from a patient with a bone related disease. The normal blood calcium concentration in a healthy adult person (both male and female) fall in the range of 8.5-10.5 mg/dL of blood. In any bone disease, hyperthyroidism or the renal disease the calcium level may shoot up to 15-20 mg/dL of blood. In well reported study it has been found that the average calcium concentration in normal healthy female was 8.5-10.5 mg/dl (19). The calcium metabolism is under the strong influence of endocrine hormones. Therefore, any abnormality in secretion of above discussed hormones affect the calcium level drastically. Female primates including human female undergoes a constant shredding and building of hormonal assisted endometrium of uterus. However, menopause state that commence in most of the female primates and human female during the age of 35-45 years. The rise and fall of endocrine hormones in the blood in different phases of menstrual cycle has a constant regulation mechanism to maintain the physiological calcium concentration (20). Therefore, direct effect on irregularity of endocrine hormones or any effect on the female reproductive cycle in turn effect the calcium level and regulatory mechanism of bone absorption and desorption. This might lead to an effect on bone density and strength of long bones (21). The sudden shutdown of several endocrine hormones related regulatory mechanisms have a direct drastic effect on the physiological calcium concentration in the blood which is required to maintain the bone health. Hence, the probability to acquire bone health related diseases like osteoporosis is more common in female with menopause started earlier. The direct effect on the physiological calcium concentration could be easily correlated with bone health, fragility and osteoporosis. However, qualitative and quantitative determination of calcium concentration is tedious and often expensive. An inexpensive biochemical/chemical laboratory assay could be easily scaled. Herein, with this inexpensive methodology a new laboratory-based method can be rigorously validated to add up in the pathological tests for identification of bone health, fracture and osteoporosis disease.

Acknowledgments

We would like to thank Faculty of Pharmaceutical Sciences, Apex Professional University for allowing us to conduct this study. I would like to thank Mr. Vijay Kumar Tilak, Faculty of Pharmaceutical Sciences, Apex Professional University, Pasighat, Arunachal Pradesh, India for his valuable support.

Authors' contributions

I, Sanjay Rawat along with our colleagues Ms. Koni Potom, Ms. Dotu Gamlin and Mr. Vijay Kumar Tilak have worked enough to shape this manuscript that highlighted the novel laboratory-based method to diagnose the osteoporosis disease in human female.

Competing interests

The authors declare no competing interest.

Data Availability Statement

Limited datasets were generated. The experimental data from human female blood sample are under the process and it will be communicated as a separate manuscript. However, materials that support finding of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate.

Approval to write and explain the hypothesis in the form of research article in creating a new knowledge domain of new chemical method for diagnosis of Osteoporosis in female has been done under the guidance of both Research and Ethics committee of Apex Professional University, Pasighat, Arunachal Pradesh, India and as per the guidelines of University Grant Commission (UGC), Government of India, New Delhi, India.

References

- 1) E. Umur, S. B. Bulut, P. Yigit, E. Bayrak, Y. Arkan, F. Arslan E. Baysoy, G. K. Can, B. Ayan, Exploring the role of hormones and cytokines in osteoporosis development, *Biomedicines* 2024, 12, 1830, 1-41.
- 2) R. Cannarella, F. Barbagallo, R. A. Condorelli, A. Aversa, S. L. Vignerra, A. E. Calogero, Osteoporosis from an endocrine perspective: the role of hormonal changes in the elderly, *J. Clin. Med.* 2019, 8, 1564, 1-14.
- 3) X. Feng, Chemical and biochemical basis of cell-bone matrix interaction in health and disease, *Curr. Chem Biol.* 2009, 3, 2, 189-196.
- 4) S. W. Hwang, J. W. Putney Jr, Calcium signaling in osteoclasts, *Biochim Biophys Acta.* 2011, 1813, 5, 979-983.
- 5) M. Miurshed, Mechanism of bone mineralization, *Cold Spring Harb Perspect. Med.* 2018, 8, a031229, 1-11.
- 6) H. C Blair, L. J. Robinson, C. L. H. Huang, L. Sun, P. A. Friedman, P. H. Schlesinger, M. Zaidi, Calcium and bone disease, *Biofactors*, 2011, 37, 3, 159-167.
- 7) A. J. Mohamed, An overview of bone cells and their regulating factors of differentiation, *Malaysian J. Med. Sci.*, 2008, 15, 1, 4-12.
- 8) T. S. Boutros, Basics of management of calcium and bone disorders, *J. Internal Med Emerg. Res.*, 2024, 5, 3, 180-203.
- 9) J. Peugh, A. Khalil, M. R. Chan, K. E. Hansen, Teriparatide treatment for hypercalcemia associated with adynamic bone disease, *JBMR Plus*, 2019, 3, 7, 1-5.
- 10) B. H. Mullin, N. J. Pavlos, S. J. Brown, J. P. Walsh, R. A. McKeller, S. G. Wilson, B. K. Ward, Functional assessment of calcium-sensing receptor variants confirms familial hypocalciuric hypercalcemia, *J. Endocrine Soci.*, 2022, 6, 1-10.
- 11) A. A. Licata, Osteoporosis, teriparatide, and dosing of calcium and vitamin D, *N. Engl J. Med.*, 2005, 352, 18, 1930-1931.
- 12) I. B. Shabat, M. H. B. Shabat, S. B. Abraham, I. Sagy, G. Tsaban, M. C. Lahav, G. Goldinger, M. Peled, C. Bartal, Reliability of blood tests taken from the peripheral intravenous catheter, *Medicine*, 2022, 101, 28, 1-7.
- 13) M. Deschaka, Blood collection in practice, a guideline for phlebotomists, *Labowarte Von A-Z*, 3rd edition, Kolhhammer Verlag, Stuttgart, Germany, 100 pages.
- 14) H. Davies, A. Jacob, L. Abou, L. Coventry, E. Jacob, Drawing blood from peripheral intravenous cannula and its effect on cannula dwell time, phlebitis and bloodstream infection: a randomized controlled study, *Collegian*, 2024, 31, 181-187.
- 15) J. W. Nieves, Osteoporosis: the role of micronutrients, *Am. J. Clin. Nutr.*, 2005, 81, 1232S-1239S.
- 16) V. Tai, W. Leung, A. Grey, I. R. Reid, M. J. Bolland, Calcium intake and bone mineral density: systemic review and meta-analysis, *BMJ*, 2015, 351, h4183, 1-14.
- 17) S. Dalemo, R. Eggersten, P. Hjerpe, E. G. Elmqvist, K. B. Bostrom, Bone mineral density in primary care patients related to serum calcium concentrations: a longitudinal cohort study from Sweden, *Scand. J Primr. health care*, 2018, 36, 2, 198-206.
- 18) G. Voulgaridou, S. K. Papadopoulou, P. Detopoulou, D. Tsoumana, C. Giaginis, F. S. Kondyli, E. Lymperaki, A. Pritsa, Vitamin D and calcium in osteoporosis, and the role of bone turnover markers: a narrative review of recent data from RCTs, *Diseases*, 2023, 11, 29, 1-26.
- 19) J. Jhang, Y. Wang, J. Guo, H. Liu, Z. Lei, S. Cheng, H. Cao, The association between ten anthropometric measures and osteoporosis and osteopenia among postmenopausal women, *Nat. Sci. Report.*, 2025, 15, 10994, 1-11.
- 20) A. Lacreus, Chennareddi, K. G. Gould, K. Hawkes, S. R. Wijayawardna, J. Chen, K. A. Easley, J. G. Herndon, Menstrual cycles continue into advanced old age in common chimpanzee (*Pan troglodytes*), *Biol. Reprod.*, 2008, 79, 407-412.
- 21) C. Higham, B. Abrahamsen, Regulation of bone masses in endocrine diseases including diabetes, *Best Pract. Res. Clin. Endocrinol. Metabol.*, 2022, 36, 101614, 1-14.

Protocol+for+determination+of+calcium+in+Osteoporosis+p...

ORIGINALITY REPORT

2%

SIMILARITY INDEX

2%

INTERNET SOURCES

2%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

1

ijpsr.com

Internet Source

1%

2

www.researchsquare.com

Internet Source

1%

Exclude quotes On

Exclude matches < 14 words

Exclude bibliography On