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

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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 bonein human body. The calcium levelis 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 testsgenerally 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.

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Introduction

Osteoporosis is a disease of bonethat 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 osteoplast 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. Thediscussed 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.

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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 osteoidorganic partof 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 bore 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 week 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 methos 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 toother 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 supernatantcontaining 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 eachlabeled test tube.
15) Compare the formation of deep blue-black color in each test tube

Figure 1

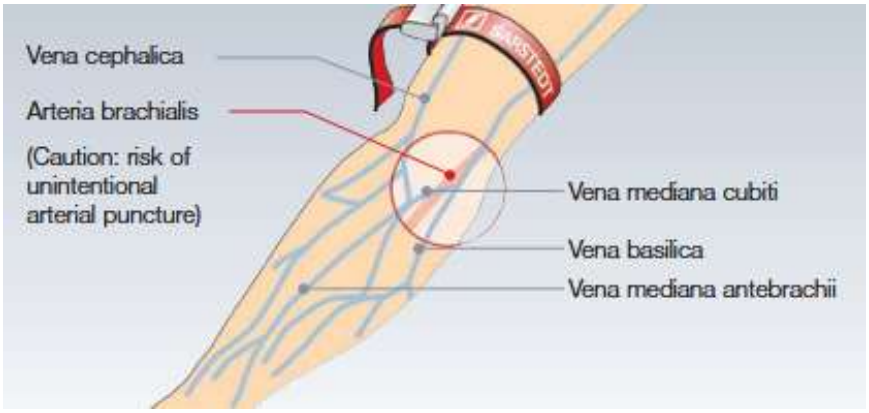

Fig.1 Point for vein puncture and blood sample collection from hand (Source- Labowarte von A-Z, 3rd edition, Kolhhammer Verlag, Stuttgart, Germany, 100 pages)

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 turnprovides 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 inmaintaining 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 15-20mg/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.

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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.

DataAvailability 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 femalehas 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.

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