BIOCHEMICAL PARAMETERS IN OSTEOPOROTIC PATIENTS[+]

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Abstract

Alkaline phosphatase activity, Calcium concentration and 17 ß-Estradiol level were measured in sera of (20 male, 18 female) .Osteoporotic patients their ages ranged from (50 to 82) years old, and (28) normal individual (control)whose ages ranged between (54 and 70) years old. The results indicate a highly significant increase ($p<0.001$) in Alkaline phosphatase activity, Calcium concentration and 17 ß-Estradiol level between patients (male and female), also between Ostesporotic patients when compared with that of normal individual. While significant increase of ($p<0.05$) in Calcium concentration when compared between female osteoporotic patients and normal individual (control).

Our aim of this study is to evaluate the relationship between Osteoporosis and the level of Alkalin Phosphatase,Serum Calcium and 17 ß-Estradiol level .

المستخلص:

تم قياس فعالية انزيم الفوسفاتيز القاعدي Alkaline phosphatase وتركيز الكالسيوم ومستوى ١٧ بيتا استراديول 17-B-Estradiol في مصل الدم للمرضى المصابين بتنخر العظام(٢٠ ذكراً و ١٨ أنثى) والتي تتراوح اعمارهم بين (٥٠-٨٢) سنة ومقارنة مع (٢٨) شخصاً طبيعياً (مجموعة التحكم) اعمارهم تتراوح بين (٥٤-٧٠) سنة أظهرت النتائج زيادة عالية ($p<0.001$) في فعالية أنزيم Alkaline phosphatase وتركيز الكالسيوم ومستوى ١٧ بيتا استراديول 17-B-Estradiol بين المرضى(ذكوراً وإناثاً) ، وكذلك بين مرضى تنخر العظام عند مقارنتهم بالأشخاص الطبيعيين. بينما أظهرت النتائج زيادة ملحوظة($p<0.001$) في تركيز الكالسيوم عند مقارنة المرضى الاناث المصابات بتنخر العظام والاشخاص الطبيعيين(مجموعة التحكم) .

Introduction

Osteoporosis is a disease characterized by low mass and structural deterioration of bone [1,2],causing increased likelihood of fractures, particularly of the hips, spine and wrist[1], and that characteristically affects postmenopausal women[3].

Osteoporosis decrease which causes in bone mass with preservation of the normal ratio of mineral to matrix primarily calcium and phosphorous[2],is a common

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abnormality that can be caused by increased bone resorption or decreased bone formation[1].

Osteoporosis is different from most other diseases or common illness in that there is no one single cause [2]. All normal humans gain bone early in life, during growth, and after a plateau begins to lose bone as they grow older[1,2]. When this loss is accelerated or exaggerated, it can lead to fractures and other complications[1], increased age is associated with a greater prevalence of osteoporosis, but heredity also plays a part, premature menopause or estrogen deficiency increases the risk of osteoporosis due to chronic conditions such as liver disease or bowel inflammation[4].

Risk factors for the development of osteoporosis include sex, small thin body frame, advanced age, a family history of osteoporosis, early menopause, amenorrhea, anorexia nervosa, low-calcium diet, use of certain medications,low testosterone levels in men ,cigarette smoking,excessive alcohol intake,and malabsorption problems[3].

Bone mass is approximately 30% higher in men than women. It is important to note however, that bone mass declines with age in all people and is influenced by sex, race, menopause and bodyweight-to-height ration[3].

Estrogen involved (estrogen, estradiol and estriol) are C_{18} steroids secreted by granulose cell of the developing ovarian follicle, corpus luteum or placenta[4].

Estrogen is also produced in by amortization of androgens in adipose tissue, liver and skin. And it is necessary to the cyclic endomderial changes, for reproduction, development of the breast, health of the skin and vascular system and bone homeostasis, so estrogen regulates the bone remodeling process[4,5].

Alkaline phosphatase is present in many tissues including liver and bone, and catalyzes the hydrolysis of phosphate from a variety of phosphate esters. Alkaline phosphatase in bone probably plays a role in calcium phosphate deposition. The main sources of serum enzyme are the hepatobilirary tree and osteoblasts[1,2].

Calcium is one of the main bones forming minerals and an appropriate supply to bone is essential at all stages of life. The total body calcium depends on that absorbed from the dietary intake and that lost from the body, the body contains approximately 1-Kg calcium, most is found in the bones where calcium is a major constituent, although the 1%, which is outside the skeleton, affects many cellular metabolic processes[6,7].

Materials and Methods:

All chemicals are of highly analytical grade.

A- Patients:

Thirty-eight osteoporotic patients who attended the out patients of Al-Sheed M.B. Al-Hakeem hospital and Al-Kadyimia teaching hospital,the diagnosis of osteoporosis is by plane X-ray. Over a period of ten months from November 2003 to August 2004.

Twenty patients were females and eighteen patients were males, their mean age was (64.3 ± 6.8) ranged from (50-82) .

Table (1) : Sex and mean age of the patients

Samples	N	Mean age ±S.D
Male	20	66.2±5.8

Female	18	62.4±7.9
Total	38	62.8±6.6
Control	28	60 ±6.2

Blood Sample Collection:

Five milliliters of venous blood samples were obtained from each patient by using 5 ml syringe and immediately transferred to a clean and sterilize plastic tubes. These samples were left for 15 min. at room temperature for coagulation. Then the serum was separated from the cell by centrifugation at 3000xg for 10 min. and was used for the same day for measurement parameters.

The sera samples should be nonhemolyzed and nonjaundice in order to avoid any interference with the obtained results. At the same time blood samples were collected from (28) healthy persons with age to be used as a control from same measurement.

B. Methods:

Instrument used in this study are:-

1- Atomic absorption spectrophotometer USA (Model 305B) with nitrous oxid-acetylene burner head(Flame type)[8] .

- This type used for Ca determination ,absorption was measured in fuel rich flame in order to obtain maximum sensitivity.

  Single element hallow cathode lamp is used as line-radiation source and is operated at current or energy recommended by mannfacturer.The conc. Of Ca is determined by comparing the signal from diluted serum with signal from aqueous calibrators.

  A hallow cathode lamp mode of Ca was used wave length as light specific for Ca is 422.67 nm, working standerd 0.1 ppm was prepared from stock standard of 1000 ppm (dissolved 1058 gm $CaCO_3$ in 10 ml HCL) , diluted in 1 liter with deionized water in polyethylene plastic container.Refrence range for serum Ca is 9-11.5 mg/100 ml=90-106 µg/ml (ppm).

- Colorimetric determination of alkaline phosphatase activity is made according to the following reaction[9,10]:

phenylphosphate $\xrightarrow{\text{Alkaline phosphatase}}$ ***Phenol+ phosphate***

The phenol librated is measured in the presence of amino-4-antipyrine and potassium ferricyanide at wave length 510 nm.

- Estrogen determination by VIDAS HCG, VIDAS HCG is an automated quantitative test for use on the VIDAS instruments[11].

  Firstly,the sample is taken and transferred into the well containing alcaline-phosphatase labeled anti-hCG antibody .The sample/conjugate

mixture is cycled in and out of the SPR several times to increase the reaction speed.The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich".

Then,the remaning free hCG sites are saturated by cycling the conjugate in the fifth well of the strip in and out of the SPR .Unbound components are eliminated during the washing steps.

During the final detection step,the substrate (4-Methyl-umbelliferyl phosphate)is cycled in and out of the SPR.The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone),the fluorescence of which is measured at 450 nm.The intensity of the fluorescence is proportional to the concentration of antigen present in the sample At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

Results :

The results presented in Table (1) show the serum Alk.phosphatase activity in control, and osteoporotic patients.

Table (1): The level of serum Alk.phosphatase (mmol/L) for control &Osteoporotic patients.

Samples	Size	S.Alk.phosphatase Mean±S.D (mmol/L)	p-Values
Control	n=28	80.2±2.22	P<0.001
Male	n=20	52.51±5.827	P<0.001
Female	n=18	77.25±3.68	P<0.001

(p<0.001) High significant
n= number of patients

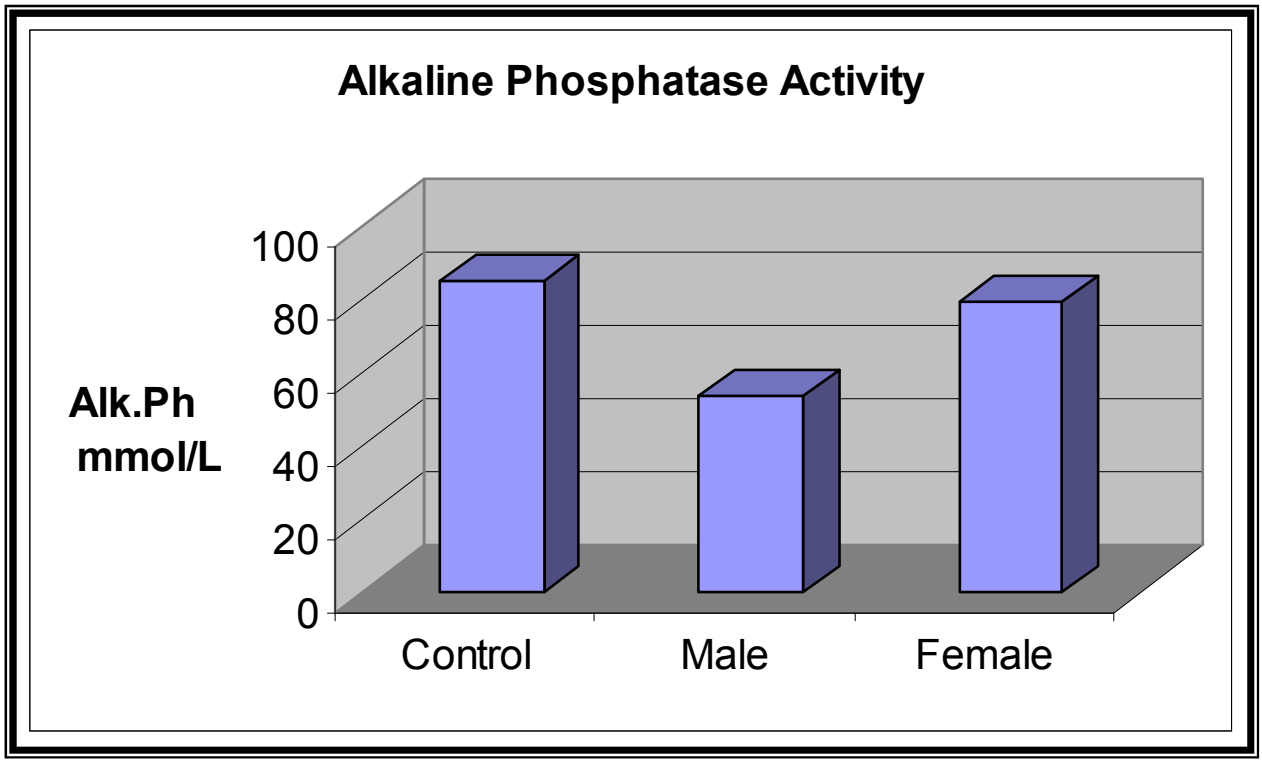

Figure (1): Determination of the level of Alk.phosphatase activity (mmol/L) for controls and osteoporosis patients.

The results presented in Table (2) show the serum Calcium concentration in control, and osteoporosis patients.

Table (2): The level of serum Calcium concentration (mmol/L) for control &Osteoporotic patients.

Samples	Size	S.Calcium concentration Mean±S.D (mmol/L)	p-Values
Control	n=28	2.62±0.1644	P<0.001,p<0.05
Male	n=20	2.356±0.129	P<0.001
Female	n=18	2.566±0.1869	P<0.05,p<0.001

P-value (p<0.001) high significant between osteoporotic male patients as compared with control and female patients, (p<0.05) significant between osteoporotic female compared with control
N = number of patients

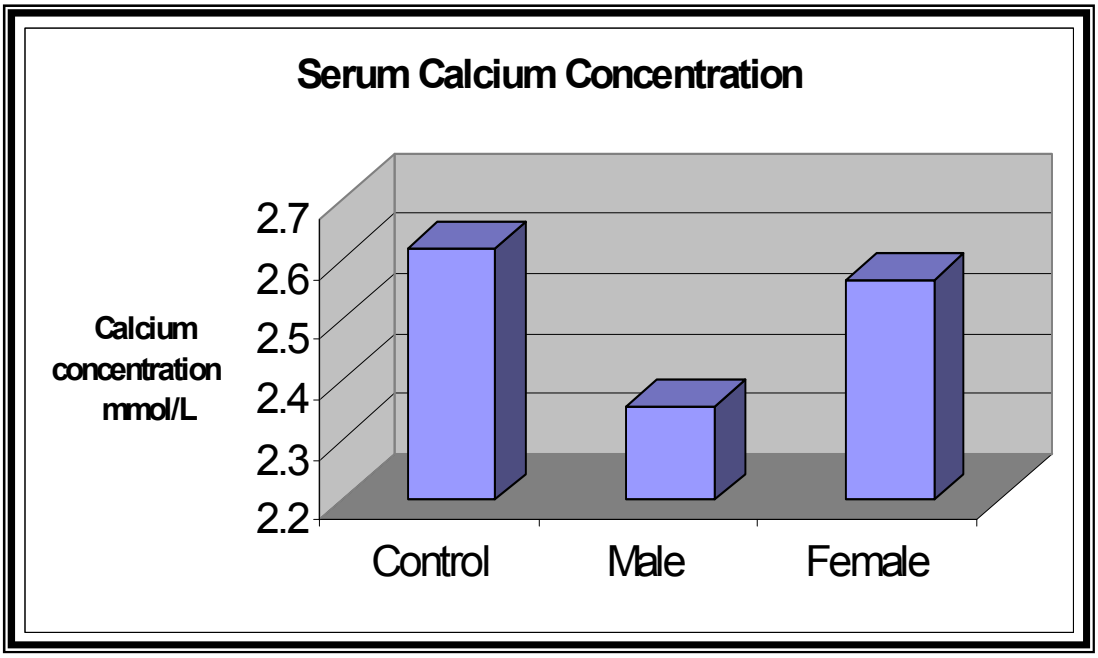

Figure (2): Determination of the level of S.Calcium concentration(mmol/L) for controls and osteoporotic patients.

Table (3): The level of serum Estradiol (pmol/L) for control &Osteoporotic patients.

Samples	Size	S.Estradiol Mean±S.D (pmol/L)	P- Values
Control	n=28	117±6.221	P<0.001
Male	n=20	48.343±13.115	P<0.001
Female	n=18	27.81±2.253	P<0.001

(p <0.001) = High significant.
N = number of patients.

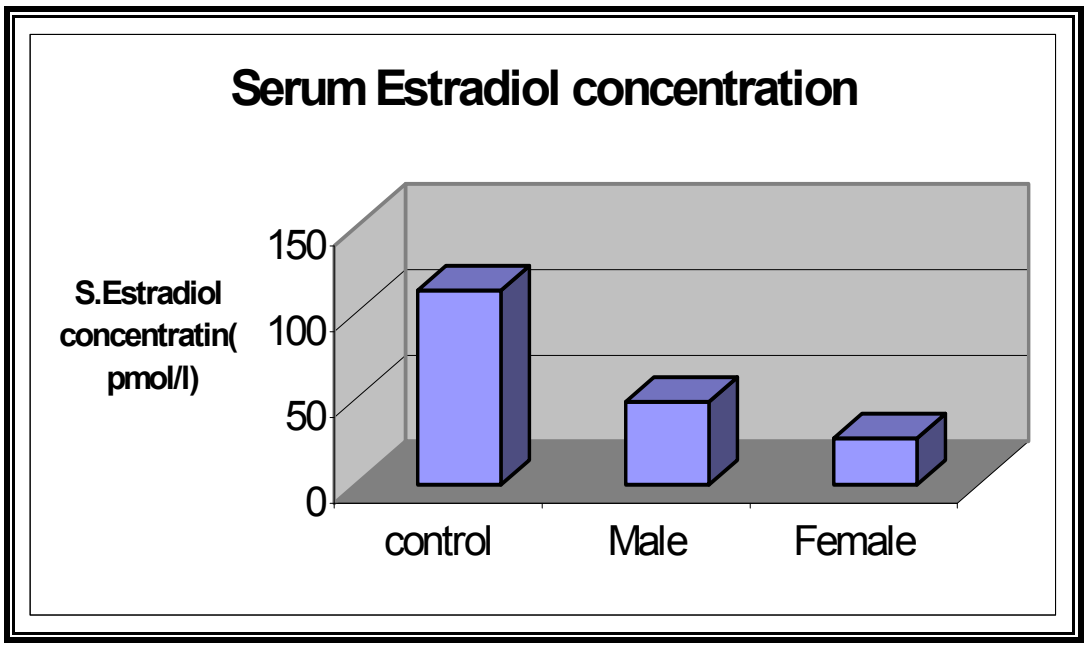

Figure (3): Determination of the level of Serum Estradiol concentration (pmol/L) for controls and osteoporotic patients.

Discussion:

Osteoporosis is a complex multifactorial disease, determined by genetic and environmental factors as well as their interactions. Alarge number of molecular, genetic and environmental factors underlying osteoporosis have been identified in past decades[12].

It is well known that serum 17ß-estradiol concentration is very low in postmenopausal women, and estrogen deficiency is important for the pathogenesis of both types of osteoporosis (in both female and male)[13] , The result presented in Table(3) shows that the level of 17ß-estradiol decreases in both male and female osteoporotic patients when compared with that of controls($p<0.001$). That estrogen deficiency is related to menopause, ameuorrhea, and low testosterone level in men increases osteoporosis[3].

Estrogen aids in slowing down loss for a few years,but progesterone is the proactive hormone because it has a stimulatory effect on the osteoblastic cells which encourages bone growth,Estrogen not only increase Bone Mineral Density(BMD),but also reduces the risk of osteoporotic fractures[14].

The results presented in Tables (1), (2) show that the biochemical tests are generally unhelpful in osteoporosis, calcium and alkaline phosphatase usually being normal, alkaline phosphatase activity in serum is usually estimated to detect increased levels. However, markedly released levels are found in the inherited condition hypophophateasia, which is caused by defective bone calcification[15] .Serum alkaline phosphatase levels are often elevated in many bone disorders including paget disease and osteomalacia.The invasion of bone by cancer from a primary tumor located elsewhere such as breast,lung or prostate) occurs by metastasis and leads to an elevation of alkaline phosphatase.Obstructive liver disease also elevates alkaline phosphatase .Morover,there is a physiological elevation of alkaline phosphatase during adolescence that is associated with growth of the skeleton[1].

Calcium deficiency is a dietary factor strongly implicated in of the cause of this disease.[3] , a deficiency of calcium which aids in the absorption of calcium into the bone can cause bone softening, increased bone loss,and increased risk of fracture[1],therefore calcium is an essential nutrient for the prevention and treatment of Osteoporosis[14].

Conclusion:

1-There is significant decrease in serum calcium in male patients with decrease activity of Alkaline Phosphatase, which is the most significant cause of osteoporosis in addition to decrease in physical activity of aged male. .

2- It is concluded that dietary deficiency of calcium may be an implemantry cause in addition the importance of estrogen especially in female

Recommendation:

The recommendation of our survey is to supplement the female patients diet by taking estrogen conjugated by progesterone and to increase dietary calcium with increase in physical activity in specialized physio therapy center for old patients.

The other recommendation is to study the drugs used as profelactid for osteoporosis and follow up them.

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