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Full Length Research Paper

Influence of aging, free radicals and weight gain since menopause on bone mineral metabolism

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Bone is a dynamic tissue that is being remodel constantly throughout life. It is composed primarily of the inorganic materials (Ca and Phosphate) and organic matrix (type-1- collagen). Bone formation is an orderly process in which inorganic mineral is deposited in relation to organic matrix. During the bone resorption first calcium and phosphorus are released into the extra cellular fluids and organic matrix is then desorbed. The concentrations of Ca, Phosphate and Mg in plasma are dependent on the net effect of bone mineral deposition and resorption, then intestinal absorption and renal excretion. Osteoporosis is the term used for diseases that cause a reduction in the mass of bone per unit volume and is one of the dreaded afflictions of ageing. There is a close relationship between estrogen deprivation and its development. In the present study, we investigate the effect of age and body weight on blood indicators of bone health namely, Ca, Mg and P. Bone mineral marker ALP was used which have more advantages when predicting the postmenopausal bone loss. The subjects were classified into three groups namely controls, premenopausal and postmenopausal. In our study blood levels of both Ca as well as Mg were found to be lowered in postmenopausal women. Mg and Fe are the major minerals that doctors find deficient in people who are overweight. Blood level of P is also decline in our study. So, that Ca: P ratios are maintained.

Keywords: Menopause, oxidative stress, ageing, mineral, enzymes.

INTRODUCTION

Bone is a dynamic tissue that is being remodel constantly throughout life. It is composed primarily of the inorganic materials (Ca and Phosphate) and organic matrix (type-1-collagen). Bone formation is an orderly process in which inorganic mineral is deposited in relation to organic matrix. During the bone resorption first calcium and phosphorus are released into the extra cellular fluids and organic matrix is then desorbed. The concentrations of Ca, Phosphate and Mg in plasma are dependent on the net effect of bone mineral deposition and resorption, then intestinal absorption and renal excretion. Parathyroid hormone (PTH) and 1, 2, 5-dihydroxy cholecalciferol (calcitriol) are the principal hormones regulating these processes[(1)]. After 40-50 years of age cortical bone is lost at the rate of about 0.3-0.5 % per year in both the sexes. An accelerated loss of cortical bone is superimposed on age related loss around menopause[(2)].

Although the reasons for overweight and obesity in women are multilayered, it is well established that menopause is associated with weight gain and an unfavorable alteration in body composition[(3, 4)]. Several studies have demonstrated that deposition of visceral adipose fat is elevated in the postmenopausal state[(5)]. Markers of bone formation and resorption have been decreases with age reaching 30-50 years. Osteoporosis is the term used for diseases that cause a reduction in the mass of bone per unit volume and is one of the dreaded afflictions of ageing[(6)]. There is a close relationship between estrogen deprivation and its

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development. Biochemical markers of bone turn over have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone diseases[7]. They reflect the whole body rates of bone resorption and bone formation. In general women lose about 1% of their bone density per year during and after menopause. Serum alkaline phosphatase (ALP) is the most commonly used marker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. The total ALP serum pool consists of several diametric isoforms which originate from various tissues such as liver, bone, intestine, spleen, kidney and placenta.

In the present study, we investigate the effect of age and body weight on blood indicators of bone health namely, Ca, Mg and P. Bone mineral marker ALP was used which have more advantages when predicting the postmenopausal bone loss.

MATERIALS & METHODS

The subjects were classified into three groups namely controls, premenopausal and postmenopausal. Those in the age group of 25-35 years are subjected under normal menstrual cycles and are taken as control group. Those in 35-45 years age range having irregular menstrual cycles are subjected to premenopausal group and women in the age group above 45 years were assigned as postmenopausal group. The age of the subjects was recorded and body weight was measured using standard techniques (Physical Status; The use and Interpretation of Anthro Pometry WHO Technical Report Series-854. Report of A WHO Expert Committee World Health Organization, Geneva, 1995). The inclusion criteria was that they had not been previously diagnosed for dyslipidemia, hypertension or hyperglycemia. Care was taken to select those subjects who were not on Hormone Replacement Therapy (HRT). The final sample that satisfied all inclusion criteria comprised of 90 females.

Biochemical Analysis

Ca, P, Mg and ALP were estimated in serum of selected subjects. Ca was estimated with Ortho-Cresoephthaleia complexions (OCPC) method which reacts with calcium to form a purple colored complex. After incubation for 5 minutes at room temperature the absorbance of standard and sample was measured against blank at 570nm.

Phosphorus was estimated by modified Gomorri's Method in the serum of selected samples. Phosphate ions in acidic medium react with ammonium molybdate to form a phosphomolybdate complex. This complex is reduced to molybdenum blue complex, the intensity of which measured after incubation at room temperature for 5 minutes against blank at 570nm.

Mg was estimated by Calmagite Method in the same serum sample that of Ca and P. A red colored complex is formed in this method from the reaction of Mg with Calmagite. The intensity of colored measures the amount of Mg in blood samples. This intensity is measured against blank at 570nm.

ALP estimated by Tris-carbonate buffer, kinetic method in the serum sample which involves the hydrolysis p-nitro phenyl phosphate to p-nitro phenol in the presens of Mg ions.

Differences between mean and standard deviation of groups were assessed using students-t-test. Odds ratios (OR) and 95% confidence intervals (CI) were obtained for bivariate analysis to asses age and body weight as risk factors for various indices of oxidative stress. Person's correlation coefficients (r) were also calculated to study their relationship. All statistical analyses were done using MS-office excel-2003 and prism -3 software.

RESULTS

The dependence of body weight, Ca, P, Mg and ALP on various reproductive stages (table-1) is indicated. Significant decrease ($p<0.05$) in Ca, P and Mg and significant increase in ALP ($p<0.001$) was found between control, premenopausal and postmenopausal groups. The median value of age of the respondents under investigation is 41 years and of body weight is 61 kg. So the sample is divided into two groups with regard to age i.e. <41years and ≥42 years and with regard to body weight i.e. <61kg and ≥61 kg. It can be seen from (table-2) that weight above 61 kg and age more than 41 years are both significant ($p<0.01$), risk factors for decrease in Ca, P and Mg and increase in ALP. The odd off decreased Ca, P, Mg and increased ALP varies between 4.5 and 29.2 (table-3). The high correlation were found between age and Ca, P, Mg and ALP (-0.89, -0.58, -0.55 and 0.94) and between weight and Ca, P, Mg and ALP (0.77, 0.47, 0.52 and 0.74) (table-4).

The effect of age is less than the effect of weight on reductions in blood Ca. However age has a greater impact than weight on blood phosphorus and ALP and the effects of age and weight on Mg are similar.

DISCUSSION & CONCLUSION

Research has shown that the weight gained in women of menopausal age is more related to the ageing process than to the hormonal changes that occur with menopause. As women aged they experience gradual decrease in muscle mass and increase in body fat. Negative thought patterns and moods that are common during the menopausal years may also contribute to weight gain. A study from the University of Pennsylvania, School of Medicine found that middle aged women who reported high levels of depressive symptoms and anxiety

Table 1. Blood levels of Calcium, Magnesium, Phosphorus and Alkaline phosphatase in females aged 25 to 55 years.

S.N.	Age (Years)	Weight (Kg)	Calcium (mg/dl)	Magnesium (mg/dl)	Phosphorus (mg/dl)	ALP (IU/L)
Female	40.6±9.62	60.3±5.55	8.23±1.36	2.09±0.35	3.10±10.38	107.4±50.8

ALP: alkaline phosphatase.

Table 2. Blood levels of Calcium, Magnesium, Phosphorus and Alkaline phosphatase in female adults belonging to different age groups.

Parameters	Control (25-35 yrs)	Age group Premenopausal (35-45 yrs)	Postmenopausal (45-65 yrs)
Age (Years)	29.8±3.08	40.4±3.16	51.7±3.98
Body weight (Kg)	54.6±2.56	60.7±2.42	65.7±4.29
Calcium (mg/dl)	9.88±0.45	8.07±0.35*	6.75±0.52**
Magnesium (mg/dl)	2.34±0.34	2.12±0.23*	1.81±0.26**
Phosphorus (mg/dl)	3.35±0.24	3.17±0.37	2.78±0.27**
ALP (IU/L)	60.05±13.05	89.5±10.3*	172.6±24.3**

***** indicates that values are statistically significantly different compared to group-1 at $p<0.05$.
****** indicates that values are statistically significantly different compared to groups-1 and 2.at $p<0.01$.

Table3. Assessment of age and body weight as risk factors for changes in blood minerals and alkaline phosphatase.

Risk factors	Calcium		Phosphorus		Magnesium		ALP	
	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Females								
Weight≥60kg	29.2	11.0-77.8	5.9	0.7-45.6	4.5	1.4-14.0	12.6	4.7-33.8
Age≥41 years	13.7	5.2-36.0	9.1	1.2-67.8	5.6	1.8-17.5	22.9	8.6-60.7

Cut-off values:
Calcium: 8.7-11.0 mg/dl
Phosphorus: 2.5-5.0 mg/dl
Magnesium: 1.3-2.5 m Eq/
Alkaline phosphatase: 15-112 IU/litre at 37 C

were more likely to experience greater amount of weight gain[8].

Blood Ca levels are very efficiently controlled within a narrow range, due to importance of ionized Ca levels in blood. The reduction in blood Ca levels with increasing age, in postmenopausal women, therefore at first seems to be surprising. However, significant decrease in total and ionized Ca has been reported in Indian postmenopausal women compared to premenopausal women[9]. Nutritional hypocalcaemia has also been reported to be a result of Mg deficiency, because parathyroid hormone release depends on Mg[10]. Pi is widely recognized to be one of the most important constituent minerals of bone. The content of P in the body increases from 4-5 gm/kg at birth to 10-12gm per kg in adulthood[11]. Previous studies in vivo and in vitro have reported that physiological regulation of proximal tubular Pi reabsorption is most likely related to capacity of apical Na/Pi co transport [12, 13, 14]. In menopause phosphate enters the circulation along with Ca. In contrast, administration of estrogen to women at menopause reads to reduction of bone resorption[15], thereby suppressing the flux of Ca and Pi into the circulation and ultimately decreasing the circulating level of Pi. However little is

Table 4. Correlation between changes in blood minerals and alkaline phosphatase with increasing age and weight.

Correlation coefficient between		Females
Age	Weight	0.78
Age	Calcium	-0.89
Weight	Calcium	-0.77
Age	Magnesium	-0.55
Weight	Magnesium	-0.52
Age	Phosphorus	-0.58
Weight	Phosphorus	-0.47
Age	ALP	0.94
Weight	ALP	0.74

known about the effects of estrogen on renal reabsorption of Pi in women.

In our study blood levels of both Ca as well as Mg were found to be lowered in postmenopausal women. Mg and Fe are the major minerals that doctors find deficient in people who are overweight. Blood level of P is also decline in our study. So, that Ca: P ratios are maintained. In this connection an increase in blood level of P have been implicated in greater bone mineral loss, through interaction of PTH, 1, 2, 5-dihydroxy cholecalciferol and intestinal Ca absorption[16]. Research has clearly established a link between osteoporosis and Mg deficiency. Although most women have heard of the need for Ca supplementation following menopause, few are aware that Mg is equally important. Infact increasing Ca without increasing Mg can actually cause more harm than good[17]. Without sufficient Mg the body loses its capacity to move K in to the cells. It is also needed to shift Ca into and out of the cell. Dr. Guy Abraham M.D. found that when Ca intake is decreased, it is utilized well than when it is present in high levels. Dr. Abraham advocates taking more Mg to correct Ca deficiency related diseases. Increasing dietary Mg often decreases menstrual cramping as well as PMS. Ca causes muscle to contract while Mg helps them to relax.

The earlier studies[18, 19] suggested the estimation of total ALP levels in serum is a useful marker in assessing mineralization activity of osteoblasts in postmenopausal women. It is well known that estrogen deficiency induces the synthesis of cytokines by the osteoblasts, monocytes and T-cells and thereby stimulates the bone resorption. Consequent to estrogen deficiency causes osteoporosis in postmenopausal women. In case of premenopausal women the sufficient oestradiol levels inhibit the synthesis of cytokines and thereby lower the bone resorption rates[20].

In our studies it was found that age and overweight are both significant risk factors for disturbances in blood levels of bone minerals and ALP. Being overweight is a greater risk than old age for reduction in blood Ca but age has a greater impact on blood P and ALP and the effect of age and weight on Mg are similar. It declines to similar extent in reaction to both.

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