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Biochemical Bone Indices and Serum Uric Acid Changes in Postmenopausal Women

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ABSTRACT

The objective of this study was to evaluate the effect of menopause on: body mass index (BMI), serum levels of total protein, albumin, globulin, total calcium, uric acid, activity of acid phosphatase (ACP) and tartarate-resistant acid phosphatase (TRACP 5b). The study involves 50 participants who were categorized into two groups; premenopausal women group (n=25) (age, 47.18 ±4.10 years) and postmenopausal women group (n=25) (age, 58.58 ±4.51years). In comparative evaluation, the serum total protein, calcium, uric acid, activity of ACP and TRACP 5b were found to be significantly increased ($p<0.05$) in post menopausal women as compared to premenopausal women. Postmenopausal women are exposed to a greater risk of serum biochemical changes as compared to the premenopausal women, thus leading to an increased risk of osteoporosis and cardiovascular diseases after menopause.

Keywords: Postmenopausal women, osteoporosis, tartarate resistant acid phosphatase, total and ionized calcium.

تغييرات مؤشرات العظم الكيموحيوية وحامض البوليك في مصل النساء بعد سن انقطاع الطمث

الملخص

تهدف هذه الدراسة لمتابعة تأثير انقطاع الطمث على: مؤشر كتلة الجسم BMI، مستويات كل من البروتين الكلي، الألبومين، الكلوبيولين، الكالسيوم، حامض البوليك، فعالية أنزيم الفوسفاتيز الحامضي (ACP) و فعالية أنزيم الفوسفاتيز الحامضي المقاوم للترترات (TRACP) في مصل الدم لنساء قبل سن انقطاع الطمث و بعده. تضمنت الدراسة 50 مشاركة تم تقسيمهن الى مجموعتين. المجموعة الأولى تضمنت نساء قبل سن انقطاع الطمث (العدد=25) (العمر 4.10± 47.18 سنة) وتضمنت المجموعة الثانية نساء بعد سن انقطاع الطمث (العدد=25) (العمر 51 .4± 58.58 سنة). وبمقارنة النتائج ظهر وجود ارتفاع معنوي ($p<0.05$) في كل من مستوى البروتين الكلي، الكالسيوم، حامض البوليك، فعالية كل من أنزيم الفوسفاتيز

الحامضي و الفوسفاتيز الحامضي المقاوم للترترات 5b في مجموعة النساء بعد سن انقطاع الطمث بالمقارنة مع مجموعة النساء قبل سن انقطاع الطمث. كما لوحظ بأن النساء بعد سن انقطاع الطمث قد يتعرضن لخطورة التغييرات الكيموحيوية والتي تؤدي إلى تزايد خطر الإصابة بمرض هشاشة العظام وأمراض القلب الوعائية.

الكلمات الدالـة: نساء في سن بعد انقطاع الطمث، هشاشة العظام، أنـزيم الفوسـفاتيز الحامـضي المقـاوم للترترات، الكالسيوم الكلي والمتأين.

INTRODUCTION

Menopause is defined as the cessation of menstruation for a period of longer than one year. It begins with changes in ovarian function and gradual decrease in the production of estrogen and other hormones (Padubidri *et al*., 2004). During menopause, women face various physiological, psychological and biochemical changes. The adverse effects of menopause are attributed to a decrease in the estrogen level, which leads to alternations in body mass index, insulin levels and also to increase the risk of hypertension, cardiovascular diseases, osteoporosis, diabetes mellitus, cancer and other degenerative changes in postmenopausal females (Burger *et al*., 2002). Osteoporosis is the term used for diseases that cause a reduction in the mass of bone per unit volume and it is one of the dreaded afflictions of aging. There is a close relationship between estrogen deprivation and its development (Ashuma *et al*., 2005). Despite its seemingly static appearance, bone is a remarkably labile tissue and bone turnover is a dynamic process which increases in postmenopausal period as a consequence of estrogen deficiency (Uemura *et al*., 2000). Bone loss occurs when the balance between formation and resorption is upset, and resorption is excessive resulting in a negative remodeling balance (Dogan and Posaci, 2002). Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease (Delmas *et al*., 2000). The rate of formation or degradation of the bone matrix can be assessed by measuring the enzymetic activity related to the bone forming or resorbing cells. Bone matrix components are released into the circulation, either by the osteoblasts or by the osteoclasts (Ashuma *et al*., 2005). Numerous studies have observed increased bone resorption in postmenopausal women and many markers of bone resorption and formation have been identified (Garcia–Perez *et al.,* 2004; Harimarayan, 2005; Suresh and Naidu, 2006). Tartrate-resistant acid phosphatase 5b (TRACP 5b; EC 3.1.3.2) was discovered from human leukocytes and named as type 5 acid phosphatase according to the electrophoretic mobility in native polyacrylamide gel (Li *et al.,* 1973). It is a unique bone resorption marker, since it is the only marker released from resorbing osteoclasts into the circulation (Janckila *et al.,* 2001). The occurrence of Osteoporosis in postmenopausal women is very common problem but there are very few Iraqi studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover. Menopause also affects calcium status (Nordin *et al*., 2004). Calcium ion is an essential component of skeleton, soft tissues and extra- cellular fluid. The skeleton contains 99% of bodies' calcium. Soft tissue and extra- cellular fluid contain about 1% of the body's calcium. There are growing

evidences for the importance of nutrition in the maintenance of bones and joints health (Qureshi *et al.,* 2010). Nutrition imbalance with endocrine abnormalities may be involved in osteoporosis (Sheweita and Khoshal, 2007). Adverse changes in serum calcium, ACP and TRACP activity, total protein and albumin due to estrogen deficiency have been implicated in the increased incidence of osteoporosis in postmenopausal women (Recker *et al*., 1998). Despite the doubling of the incidence of gout among women over the past twenty years (Arromdee *et al.,* 2002) and the substantial prevalence particularly in the aging female population (Kramer and Curhan, 2002), little is known about the risk factors for gout and hyperuricaemia specifically among women. Elevated circulating serum uric acid concentrations may be linked to an increased risk of coronary heart disease (CHD) (Simon *et al.,* 2006).

The aim of this study was to evaluate the effect of menopause on TRACP activity as one of bone resorption markers, and the status of calcium and uric acid; to study the possibility of using these biochemical parameters to early diagnose of osteoporotic changes in the normal menopausal women by easy colorimetric methods to avoid the risk of osteoporosis, fractures and coronary heart diseases.

MATERIAL AND METHODS

Subjects

This study was carried out at the Chemistry Department, College of Education for Girls/ Mosul University. The study was conducted on 50 apparently healthy females selected from the general populations, out of which 25 were of premenopausal age, (mean=47.18±4.10 years) with regular menstruation, and 25 were of postmenopausal age (mean=58.58±4.51 years) with natural menopause, permanent cessation of the menstrual cycle for more than one year. A narrow range of age group was taken to reduce the effect of aging as it is a factor affecting the bones and the measured parameters. All the selected subjects were healthy and were not suffering from any diseases like hypertension, diabetes mellitus, rheumatoid arthritis and malignancy. Also none of the subjects was on any medical treatment. The height and weight of subjects were measured and used in calculating Body Mass Index (BMI Kg/m^2).

Samples collection

For assessment of various parameters, (5 ml) of venous blood samples were collected from each subject, without tourniquet, under aseptic conditions and after overnight fasting. Samples were centrifuged at (2000xg). The obtained sera samples were estimated directly for enzyme activities or frozen at -20 °C for subsequent analysis. Hemolyzed samples were discarded.

Methods

The activity of total acid phosphatase (ACP) and tartarate-resistant acid phosphatase (TRACP 5b) were determined using 4- nitrophenol phosphate (4-NPP) as substrate (Lau *et al*., 1987; Janckila *et al*., 2001). Total protein (TP) concentration was determined by Biuret method (Burtis and Ashwood, 1986) using kit manufactured by Biolabo (France). Albumin (alb) concentration was determined by dye-binding method (Doumas *et al.*, 1971) using kit

manufactured by Biolabo. The concentration of the globulin was determined by using the following equation:

$$C_{glob}\ (g/dl) = C_{TP} - C_{alb}$$

Calcium concentration was determined by CPC method (ortho-Cresol Phthalein Complexone method) (Connerty and Briggs, 1966) using Biolabo kit (France).
Corrected total calcium (mmol/L) = measured calcium (mmol/L) + 0.02 (40-measured albumin g/L).

Uric acid concentration was determined according to uricase method (Artiss and Entwistl, 1981) by using Biolabo kit (France).

Statistical analysis

Statistical analysis was carried out using Student's paired *t*-test. The data were expressed as Mean±SD and (P) values < 0.05 were taken as significant.

RESULTS AND DISCUSSION

Table (1) shows the Mean±SD value of age, body mass index, total protein, albumin, and globulin. The results of this study showed that the postmenopausal women have a non-significant decrease (P>0.05) in BMI as compared to premenopausal women. Total protein was significantly increased (P<0.01) in post menopausal women when compared to premenopausal women. There was no significant difference (P>0.05) in the albumin and globulin values of both groups.

Table 1: Mean value of age, body mass index (BMI), total proteins, albumin, globulin in premenopausal and postmenopausal women

Variables	Pre-menopausal Women (n=25)	Post-menopausal Women (n=25)	(P) value
Age (years)	47.18±4.1	58.58±4.51	
BMI (kg/m^2)	33.02±4.37	31.95±4.6	0.36
Total protein (g/dl)	6.75±0.40	7.43±0.42	0.001*
Albumin(g/dl)	3.91±0.57	4.23±0.35	0.06
Globulin (g/dl)	2.83±0.55	3.08±0.47	0.32

- Statistically significant (P≤0.001)

Menopause is associated with numerous physiological and biochemical changes. Results from our study have shown that body mass index (BMI) has a non significant decrease. According to World Health Organization (WHO) criteria (Qureshi *et al*., 2010), pre-menopausal women were obese (BMI>30 kg/m^2) and also were postmenopausal women with little decrease. This was attributed to number of years since menopause and advancing age, since aging is often associated with loss of height, weight and development of stooped posture (Usoro *et al*., 2007). Low BMI has been known to be a risk factor for osteoporosis.

Overall body protein status is usually assessed through the levels of total protein and albumin in serum. The results of our study showed that the total protein was elevated in

postmenopausal women as compared to premenopausal women. However, serum total protein was relatively higher in menopausel women which confirms previous findings (Javed *et al.,* 2000, Ashuma *et al.,* 2005 and Kumari *et al.,* 2010), and it was attributed to aging and metabolic processes affected by menopause. The elevated total serum protein levels may also indicate dehydration, high protein and high calorie diet, liver/biliary dysfunction … etc. (Kumari *et al.,* 2010). Albumin concentration showed a non significant increase in postmenopausal women as compared to premenopausal women and it is well known that albumin concentration contributes significantly in alternation of serum total protein level (Javed *et al.,* 2000).

Results in table (2) showed the presence of a significant increase in the activity of acid phosphatase (ACP) and tartarate resistant acid phosphatase (TRACP 5b) in postmenopausal women as compared to premenopausal women. The mean level of total calcium was significantly increased in postmenopausal women compared to premenopausal women. High significant increase was observed in the mean level of uric acid of postmenopausal women compared to premenopausal women.

Table 2: Mean value of ACP and TRACP activity, total calcium, corrected calcium and uric acid levels in premenopausal and postmenopausal women.

Variables	Pre-menopausal Women (n=25)	Post-menopausal Women (n=25)	(P) value
ACP (IU/L)	5.98±3.88	9.62±3.8	0.032*
TRACP (IU/L)	2.91±1.72	5.11±2.06	0.008**
Total Calcium (mmol/l)	2.19±0.31	2.64±0.54	0.022*
Corrected calcium (mmol/l)	2.28±0.48	2.59±0.53	0.09
Uric acid (mg/dl)	3.73±0.84	5.47±1.40	0.0001**

*Statistically significant (P<0.05), **Statistically significant (P<0.01)

Biochemical parameters can give an idea about the rates of bone formation and resorption. The results revealed a significant increase in ACP and TRACP 5b activities in postmenopausal women when compared to premenopausal women and this is in accordance with many studies (Dogan and Prosaci, 2002; Rico *et al*., 2002; Suresh and Naidu, 2006) that in early menopausal women there is a high acid phosphatase activity as a result of the inhibitory effects of estrogen on bone turnover rate which is dependent on age. It is well known that estrogen deficiency induces the synthesis of cytokines by the osteoblasts, monocytes and T-cells and thereby stimulates bone resorption by increasing osteoclastic activity (Esbrit, 2001). Estrogen exerted also a major effect on bone remodeling by inhibiting interleukin (IL)-6 production that reduces bone resorption and also controls the timing of osteoclast apoptosis (Garneo and Delmas, 2004). This upsets the normal balance between bone resorption and bone synthesis. The increase in osteoclast activity is accompanied by an increase in the synthesis and secretion of TRACP 5b (Halleen *et al*., 2000). In general, women lose about 1-5% of their bone density per year during and after menopause (Kumari *et al*., 2010), that contributes to the development of osteoporosis. Estrogen inhibits bone resorption and this may justify the influence of this hormone on osteoclastic activity. So, early detection of bone loss by measuring TRACP 5b activity helps

to confirm the diagnosis of osteoporosis and assesses the future risk of osteoporotic fractures.

The results in Table (2) showed a significant increase in total calcium and this agrees with other studies (Ashuma *et al.,* 2005; Suresh and Naidu, 2006; Usoro *et al.,* 2007). Calcium ion is an essential structural component of the skeleton. Extracellular calcium ion concentration is determined by the interaction of calcium absorption from the intestine, renal excretion of calcium and bone uptake and release of calcium (Sheweita *et al.,* 2007). In postmenopausal women, estrogen deficiency may induce calcium loss by indirect effects on extra skeletal calcium homeostasis. Parathyroid hormone causes bone resorption and helps to maintain blood calcium levels. Levels of serum parathyroid hormone increased progressively with age in women due to estrogen deficiency, and so correlate significantly with the increase in bone turnover (Qureshi *et al.,* 2010). Intestinal calcium absorption also, decreases in postmenopausal women (Nordin *et al.,* 2004). All these factors are leading to increase circulating levels of calcium ion and that was obvious in the results of this study. A recent study found an independent association between the higher baseline serum calcium levels and the higher rate of cardiovascular events (Slinin *et al.,* 2011). Because calcium is predominantly transported bound to serum proteins, total calcium levels are greatly influenced by protein concentration especially albumin (Sava *et al.,* 2005).

The quality of life of women after menopause is one of the key health issues today (Agrawal *et al.,* 2009). There are growing evidences for the importance of nutrition in the maintenance of bones and joints health. Diet has been proven to be an independent risk factor for the development of osteoporosis. High protein diet (non-vegetarian diet particularly) leads to excessive acid formation which may contribute to dissolution of bones as the body tries to buffer the extra acid. Acidosis may also increase osteoclastic function directly (Ashuma *et al.,* 2005). Studies have shown that postmenpausal women with high total protein intake and high animal/vegetable protein ratio have a higher incidence of fractures (Sellmeyer and stone, 2001). High animal protein intake also induces high uric acid level.

Results in Table (2) showed a highly significant increase in uric acid level in postmenopausal women as compared with premenopausal women. This agrees with the study of (Hak and Choi, 2008) who indicat that menopause was associated with a higher serum uric acid level. The increases in serum uric acid in postmenopausal women may result from the changes in metabolism as a consequence of the menopause (Callums *et al*., 1998). Estrogen may help the kidney to excrete uric acid, so after menopause, a women's uric acid level begins to increase. It usually takes several years for the uric acid level to reach the point where crystals can form. Elevated circulating serum uric acid level may be linked with an increased risk of coronary heart disease (CHD) (Simon *et al.,* 2006).

In conclusion, this study shows an increase in the activity of both, ACP and TRACP 5b, and an increase in total calcium level in postmenopausal women which indicates that postmenopausal women are more prone to osteoporosis and fractures. Also the study indicates that menopause is associated with higher serum uric acid level which may be linked with an increased risk of coronary heart diseases.

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