Variation of Serum Osteocalcin Levels With Age and Sex in Healthy Subjects

Sağlıklı Kişilerde Yaş ve Cinsiyete Göre Serum Osteokalsin Düzeyleri

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Abstract

This study was effectuated to determine if osteocalcin levels change according to different physiological states, age or sex. A total of 250 normal subjects (122 males and 128 females; aged 0-80) were studied. Statistical analysis showed significant differences ($p<0.01$) for the following groups: 11-20 yrs. male and 11-20 yrs. female, 0-20 yrs. male and 21-80 yrs. male, 0-20 yrs. female and 21-50 yrs. female, and 21-50 yrs. female versus 51-80 yrs. female. Alkaline phosphatase levels for the age groups 0-20 were found to be higher than the age groups 21-80. On the other hand, calcium concentration did not change significantly with age. In conclusion, it can be stated that puberty and menopause cause fluctuations in osteocalcin levels.

Key words: osteocalcin, alkaline phosphatase, calcium

Özet

Bu çalışma osteokalsin düzeylerinin yaş, cinsiyet ve farklı fizyolojik durumlara göre değişip değişmediğini saptamak için gerçekleştirildi. Toplam 250 sağlıklı kişide (0-80 yaşları arasında; 122 erkek ve 128 kadın) çalışıldı. İstatistiksel analizler sonucunda aşağıda belirtilen gruplar arasında $p<0.01$ düzeyinde anlamlı bir farklılık gözlendi: 11-20 yaşlarında erkek ve 11-20 yaşlarında kadın denekler arasında, 0-20 ve 21-80 yaş erkek grupları arasında, 0-20 ve 21-50 yaşlarındaki kadın denekler arasında, 21-50 ve 51-80 yaşlarında kadın denekler arasındadır. Alkalen fosfataz düzeyleri heriki cinsiyet grubu için de, 0-20 yaş grubunda, 21-80 yaş grubuna göre daha yüksek olarak bulunmuştur. Diğer yandan, kalsiyum konsantrasyonlarının yaş ile anlamlı bir şekilde değişmediği gözlenmiştir. Sonuç olarak, puberte ve menapozun osteokalsin düzeylerinde bir değişmeye neden olduğunu söyleyebiliriz.

Anahtar sözcükler : osteokalsin, alkalen fosfataz, kalsiyum

INTRODUCTION

Osteocalcin (OC; also called Bone Gla Protein or BGP) is a 49-amino acid, vitamin K dependent, calcium- binding bone matrix protein which circulates in blood (1-6). The presence of gamma-carboxy glutamic acid (Gla) residue confers a high affinity for hydroxyapatite to the protein (7). OC is specific for bone and it is synthesised by osteoblasts and released into the circulation where it can be measured by radioimmunoassay (1,5,8). Price et. al. (9) reported that normal males had higher osteocalcin levels than women, and that osteocalcin fell with age in women. Delmas et. al. (10) have reported that osteocalcin levels increased with age in women. Epstein et. al. (11) found that serum osteocalcin levels increased with age in both sexes, and were higher in women than in men at all ages.

The aim of this study was to determine serum OC

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Biyokimya Dergisi
Ekim-Kasım-Aralık/ 1999
Sayı : 4, Cilt : 24

levels in normal subjects of different sex, age and physiological states. In addition, any possible correlation with alkaline phosphatase or calcium levels was also investigated.

MATERIAL AND METHODS

Subjects

A total of 250 normal subjects (122 males and 128 females; aged 0-80) were studied. They were divided into 8 groups by decades according to their age (Table I). The female group was also divided into two subgroups named "premenopausal (n=25)" and postmenopausal (n=38)". All subjects had normal renal function and no evidence of calcium, phosphate abnormality or bone disease, according to their history and physical and biochemical examinations. They were not receiving any medication known to affect bone tissue.

Table I: Distribution of subjects according to age and sex

Age groups	Males (n)	Females (n)
0 – 10	15	15
11 – 20	15	15
21 – 30	16	19
31 – 40	20	16
41 – 50	15	16
51 – 60	15	20
61 – 70	13	15
71 - 80	13	12

Methods

Fasting blood samples were taken in the morning and sera were separated no later than 1 hours after sampling and stored at –20 °C until analysed. Serum osteocalcin was measured by a double-antibody radioimmunoassay (CIS Biointernational / France) similar to that described by Price and Nishimoto (9).

The reagents used in this method were:

Standard bovine osteocalcin lyophilised in borate buffer containing human albumin (serial dilutions from 60 to 1 ng/ml were performed for the reference curve).

Purified bovine osteocalcin labeled 125I. The radioactivity was approximately 2 °Ci.

Rabbit serum anti-bovine osteocalcin as the first antibody.

Precipitating complex as the second antibody consisting of an insoluble complex of sheep anti-rabbit gammaglobulins and non- immunised rabbit gamma-globulins and polyethylene- glycol.

This RIA employed an overnight incubation at 2-8 °C and 15 minutes incubation followed by centrifugation and supernatant discordance for the separation of bound/free phases. The RIA sensivity was 0.35 ng/ml (min. detectable amount). The "within-assay" and "between-assay" reproducibility values, expressed by coefficient of variation (CV%), were 3.7 and 4.4%, respectively. The OC concentrations used for the coefficient of variation experiments were in the 3-15 ng/ml range.

Serum alkaline phosphatase levels were measured spectrophotometrically with p-nitrophenylphosphate as substrate (Sigma Chemical Co., St. Louis, MO). The reaction mixture has the composition: 4-nitrophenyl phosphate, 14 mmol/L; 2-amino-2-methyl-1-propanol (AMP), 750 mmol/L; Mg(II), 0.1 mmol/L; pH=10.3 at 30°C Total serum calcium was determined using the autoanalyzer (DACOS) with Coulter Dart Calcium Reagent System.

Student's-t test was used for statistical analysis.

RESULTS

Figure 1 demonstrates the changes in serum OC, as a function of age and sex. Serum OC levels are

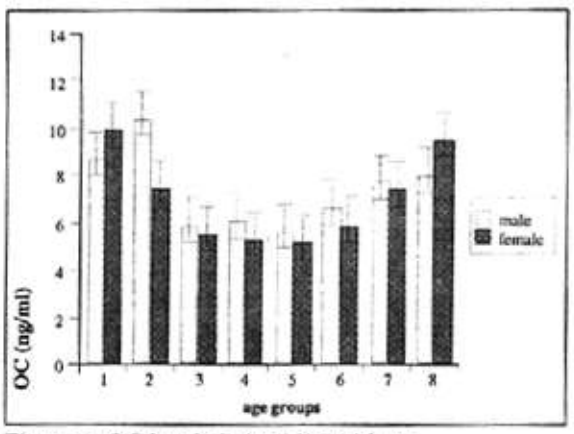

Figure 1. OC levels in healthy subjects

higher in children than in adults and increased in elderly normal subjects. The serum levels of OC were : 8.63 ± 1.7 ng/ml and 10.13 ± 1.4 ng/ml in males and females, aged 0-10, respectively, with no significant difference between the two sexes. However, a sig-

nificant difference ($p<0.01$) existed between males and females aged 11-20 (11.06 ± 1.0 ng/ml; 7.27 ± 0.57 ng/ml), with higher values in men.

Females aged 21-50 revealed a significant decrease (4.37 ± 0.42 ng/ml) in comparison with females aged 0-20 (8.7 ± 0.8 ng/ml) ($p<0.01$) and females aged 51-80 (6.97 ± 0.61 ng/ml) ($p<0.01$). However, no significant difference was found between females aged 0-20 and 51-80. Men aged 0-20 showed remarkably higher levels (9.84 ± 0.77 ng/ml) in comparison with men aged 21-80 (6.40 ± 0.38 ng/ml) ($p<0.01$).

Alkaline phosphatase levels for the age groups 0-20, (226.5 ± 23.2 U/L) were found to be higher than the age groups 21-80 (74.53 ± 2.86 U/L). On the other hand, calcium concentrations did not change significantly with age.

DISCUSSION

OC is a vitamin K-dependent bone protein of 5800 daltons synthesised by osteoblasts (12-14). According to some authors (1,10) its levels increases with age and the increase is especially remarkable in women. Moreover, Delmas (10) found higher levels of OC in children. In this study, we showed that higher serum OC concentrations were found in both sexes, aged 0-20 years, compared with all the other ages in parallel with the fact that OC rises during linear growth (15,16). In females aged 21-50, a significant decrease in serum OC was found when compared with females aged 0-20 and 51-80. There was also a significant decrease in men aged 21-80 compared with those aged 0-20. No significant difference was observed between the two sexes aged 0-10.

In our study, a pronounced decrease in OC serum levels was observed in females between ages 41-50, followed by a rise with age; this decrease has been reported to take place in other age intervals by some other authors (1,5,17,18) also followed later on by a rise in OC levels. This fall in OC can be explained by a critical physiological decrease in bone formation and by bone loss occurring in women during and after menopause. In contrast, the subsequent increase in serum OC in females aged 61-80 might suggest a recovery of osteoblastic activity due to the physiological increase in serum parathormone levels occurring in older females (1). This hormone can indirectly stimulate osteoblasts.

On the other hand, Gla residues impart upon osteocalcin its characteristic binding property and promote its interaction with hydroxyapatite. The adsorption affinity of osteocalcin for hydroxyapatite may be an important factor in the mineral dynamics of bone (3,19). If decarboxylated protein is produced, then a smaller amount of the protein will bind to the bone and a larger proportion will be liberated to the circulation. The finding that osteocalcin isolated from men in the seventh decade showed only two Gla residues per molecule, may relate to a deficiency in the carboxylating system in the elderly and explain the increasing levels of serum osteocalcin in normal ageing individuals (20).

Reports on age-related changes are controversial. This variation in literature probably reflects heterogeneity in levels of bone turnover between study population, but may also be due to different specificity and sensitivity of the antibodies used in various RIA's (1,7,18,21).

Our study has also included alkaline phosphatase (ALP) and calcium determinations, these two parameters being expected to reflect osteoblastic activity. Epstein et. al. (11) and Delmas et. al. (10) reported a correlation between alkaline phosphatase and osteocalcin levels in normal women, contrary to Price et. al. (9)'s findings, where no such correlation was observed. In our study, ALP did not show fluctuations parallel to those of osteocalcin ($r = 0.6$) ; the only age group where ALP showed high values compared with other age groups, was between 0-10 years, reflecting high osteoblastic activity in parallel with OC. The absence of correlation may be due to in part to the fact that the alkaline phosphatase levels is only a rough index of osteoblastic activity, since non-osseous tissues contribute to the serum pool of this enzyme (12,22,23). On the other hand , serum calcium levels did not change in any of the age groups (23-25).

In conclusion, it can be stated that serum osteocalcin levels change with age and sex. The most remarkable decrease occurs after puberty for both sexes and during menopause for women, while both sexes. In females higher OC levels were detected in 71-80 year age groups. In addition, the finding that pre-pubertal growth seems to be associated with increased circulating osteocalcin levels has, for the first time been presented in this study.

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