



Original Article

Usefulness of ceruloplasmin testing as a screening methodology for geriatric patients with osteoporosis

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Abstract. [Purpose] To evaluate serum ceruloplasmin levels in geriatric patients with osteoporosis. [Subjects and Methods] Seventy geriatric patients over 65 years of age were recruited. Patients were divided into two groups: group 1 ('OP', n=35) consisted of patients with osteoporosis, and group 2 (n=35) consisted of patients without osteoporosis. Dual-energy X-ray absorptiometry scanning was used in the measurement of bone mineral density in all cases. Inflammatory parameters, including C-reactive protein, sedimentation rate, and serum ceruloplasmin levels were analyzed in blood samples. [Results] No statistical differences in inflammatory parameters were observed between the two groups, however, serum ceruloplasmin levels were significantly higher in group 1 than in group 2. In Pearson analysis, serum ceruloplasmin levels were not found to be correlated with any biochemical parameters. Receiver operator characteristic curve analysis revealed that serum ceruloplasmin levels were predictive of osteoporosis with 85.7% sensitivity and 85.7% specificity over the level of 830.15. [Conclusion] Our study demonstrated that measurement of serum ceruloplasmin levels may have potential as a screening methodology for geriatric patients with osteoporosis.

Key words: Ceruloplasmin, Osteoporosis, Screening test

(This article was submitted Sep. 9, 2015, and was accepted Oct. 16, 2015)

INTRODUCTION

Osteoporosis (OP) is defined as a systemic skeletal disease characterized by bone fragility resulting from low bone mass and deterioration of bone microstructure. Etiologically, osteoporosis is divided into three groups: primary type 1, primary type 2, and secondary. The form of osteoporosis most common in women, following menopause is referred to as primary type 1 or postmenopausal osteoporosis. Primary type 2 osteoporosis, or 'senile osteoporosis', occurs after the age of 75 years and is observed in both women and men at a ratio of 2:1. Secondary osteoporosis may occur at any age and affects men and women equally. Often a consequence of various chronic inflammatory diseases, secondary osteoporosis manifests through bone loss resulting from direct effects of inflammation, poor nutrition, reduced lean body mass, immobility and the effects of treatments, especially glucocorticoids. Osteoporosis is a primary comorbidity issue for patients of chronic inflammatory diseases, especially rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, and chronic obstructive pulmonary disease^{1–10)}.

Following the discovery of the RANK/RANKL/OPG axis, primary osteoporosis has been considered to be a low grade

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chronic inflammation, mediated by increased T and B lymphocyte activity as a product of aging and estrogen deficiency^{11–13}). Inflammatory cytokines IL-1 and TNF α , produced by lymphocytes and M-CSF, have long been associated with osteoclastic bone loss, with promotion of RANKL production, through reduction of OPG production and up-regulation of the RANK receptor on osteoclast precursors, thus increasing their sensitivity to prevailing RANKL concentrations and directly stimulating differentiation of osteoclast precursors^{14–16}). IL-1 and TNF α have long been implicated in osteoclast formation in postmenopausal osteoporosis and in ovariectomized animal models^{17–22}).

Ceruloplasmin (CP) is an acute phase reactant and antioxidant, characterized by ferroxidase activity and increases in inflammation. Blood serum CP levels are correlated with presence of inflammatory diseases such as chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, Behcet's Disease, nasal polyps, cystic echinococcus, and obesity^{23–26}).

In light of this information, we designed this study to investigate the acute phase reactant and inflammatory parameters available in the diagnosis and monitoring of osteoporosis. Therefore, we aimed to evaluate CP and C-reactive protein (CRP) levels in geriatric patients with osteoporosis.

SUBJECTS AND METHODS

Patients who were over the age of 65 years and were admitted to the Harran University School of Medicine were included in this cross-sectional study. Written consent to participation was received from each patient, with the hospital's ethics committee granting approval in conformation with the principles of the 2nd Declaration of Helsinki.

Subsequently, 70 geriatric patients meeting the study criteria were divided into two groups; group 1 (n = 35) comprised patients with primary osteoporosis and group 2 (n = 35) comprised healthy patients enrolled during routine check-ups, without osteoporosis but exhibiting demographic characteristics similar to those of group 1. The same physician examined all the patients to prevent inter-observer variability. The exclusion criteria were as follows: concurrent diagnosis of endocrine diseases such as thyroid, diabetes mellitus, prolactinoma, Cushing syndrome, and hyperparathyroidism, thought to cause osteoporosis; diagnosis of chronic inflammatory disease (according to their medical history and physical examination); simultaneous prescription of osteoporosis treatment steroids, diuretics, anticoagulants, LH-RH agonist, anticonvulsants, pioglitazone, heparin, or methotrexate; on-going antacid therapy containing aluminum; presence of kidney, liver, and celiac disease; a history of gastrectomy operation, malabsorption, or total parenteral nutrition; immobility; current smoking or regular alcohol consumption; and a history of early menopause.

Body mass index (BMI) was calculated for each patient by dividing the body weight by the square of the height. Subject bone density was measured using DEXA, with T scores in the L2–L4 vertebrae and femoral neck under -2.5 considered as osteoporosis. A Hologic QDR 4500 A DEXA (Hologic INC 02154-USA) device was used in bone mineral density (BMD) measurements of patients. BMD of the L1–L4 vertebrae, femoral neck, and total femur density was expressed as g/cm², with the L1–L4 average used as the expression of the vertebral BMD.

Serum C-reactive protein, sedimentation rate, and CP levels were analyzed in venous blood samples. Five milliliter blood samples were taken from the forearm venous blood vessels of subjects and placed in Eppendorf tubes. The tubes were centrifuged at 1,500 rpm for 10 minutes to obtain serum samples for the measurement of defined parameters. All serum samples were stored at -80 °C, after labeling in a biochemistry laboratory, until the day of the analysis. The enzymatic activity of CP was measured according to Erel's method²⁴). Using this assay, ferrous ion is oxidized to ferric ion via CP ferroxidase activity. The results are expressed as units per gram protein (U/L). Glucose, sodium, potassium, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, albumin, alkaline phosphatase, calcium, phosphorus, magnesium, triglycerides, total cholesterol, high density lipoprotein-cholesterol and low density lipoprotein cholesterol (other biochemical parameters such as c-reactive protein, free T3, free T4, adrenocorticotrophic hormone, cortisol, parathyroid hormone, glycosylated hemoglobin) were analyzed using a Roche Cobas Integra 800 auto analyzer (Roche) and commercial measurement kits.

SPSS 18.0 was used in all statistical analyses (Chicago, IL, USA). The one sample Kolmogorov-Smirnov test was used to verify the normality of data distributions. Results are expressed as mean \pm SD. The χ^2 test was used for categorical variables. Independent sample T test was used to analyze parametric numerical data, and the Mann-Whitney U test used to analyze non-parametric data. Pearson correlation coefficients were used to determine correlations between serum CP levels and defined parameters measured in osteoporotic patients. Binary logistic regression analysis was performed to identify independent predictors of osteoporosis. ROC-curve analysis was implemented to find indicatory cut-off values for serum CP levels in osteoporosis patients. Values of $p < 0.05$ were considered statistically significant for all results.

RESULTS

Patient clinical, anthropometric, and biochemical findings are shown in [Table 1](#). Statistically significant differences were not observed between the groups in terms of age or gender ($p > 0.05$). Although the CRP level and erythrocyte sedimentation rate of group 1 patients were not different from those of group 2 patients ($p > 0.05$), the serum CP levels of group 1 were found to be significantly higher ($p < 0.001$). In Pearson correlation analysis, serum CP values were not associated with any observed parameter (for all; $p > 0.05$).

Binary logistic regression analysis revealed that CP levels were an independent factor ($B = 0.022$, $SE = 0.005$, $Wald = 17,816$, $p < 0.001$). In ROC-curve analysis, serum CP levels above 830.15 U/L were shown to provide 85.7% sensitivity and 85.7% specificity in the diagnosis of osteoporosis patients (Area under the curve = 0,897, 95% confidence Interval = 0,825–0,970; $p < 0.001$).

DISCUSSION

This is the first study to investigate serum CP levels in geriatric patients, and has shown that CP levels are higher in primary osteoporotic patients than in those without osteoporosis. Further, it has been shown that CP levels above 830.15 U/L can be used for the diagnosis of osteoporosis with 85.7% sensitivity and 85.7% specificity.

CP is known as an acute phase reactant and an antioxidant parameter in inflammatory diseases, and has been observed in elevated levels in several chronic inflammatory diseases^{23–26, 28–31}. In a study measuring inflammatory marker protein levels in elevated plasma inflammation, CP levels were shown to be associated with an increased incidence of chronic obstructive pulmonary disease requiring hospitalization²³. In a study on copper levels in subjects with rheumatoid arthritis, serum copper and CP levels were correlated with presence of the inflammatory process²⁴. Another study in trace elements and some extracellular antioxidant proteins levels revealed serum copper and CP levels were significantly higher in the serum of patients with systemic lupus erythematosus²⁷. CP levels were correlated with other inflammatory parameters in some of these studies.

Chronic inflammation is related to the development of secondary osteoporosis. Additionally, osteoporosis is observed concomitantly with a number of chronic inflammatory diseases; a consequence of cytokine action on the bone remodeling system in the favor of bone resorption^{1–7}. For example, osteoporosis in patients with COPD has been observed in previous studies, with osteoporosis found to be a consequence of chronic inflammation^{4–7}. Previous studies investigating osteoporosis in patients with rheumatoid arthritis and ankylosing spondylitis have shown that osteoporosis was correlated with activation of disease, low BMI, age and increasing disease duration^{1, 2}.

Alternatively, there have been studies evaluating CRP levels and oxidative status in primary osteoporotic subjects without chronic inflammation. In some of these studies, CRP levels were found to be higher, implicating that it may be possible to use CRP in screening tests for osteoporosis^{32–35}. In another study, total oxidant status, although not correlated with BMD,

Table 1. General demographics, clinical data, and biochemical parameters of the patients

	Group 1 (n=35)	Group 2 (n=35)
Age (years)	70.0 (65.0–83.0)	70.00 (65.0–84.0)
Gender (F/M)	28/7	21/14
BMI (kg/m ²)	28.1 ± 3.4	28.2 ± 3.3
Glucose (mg/dL)	107.6 ± 9.2	105.3 ± 13.2
Urea (mg/dL)	1.1 ± 0.2	1.2 ± 0.2
Creatinine (mg/dL)	0.7 (0.5–1.1)	0.7 (0.4–1.2)
AST (U/L)	19.6 ± 6.4	21.2 ± 8.0
ALT (U/L)	18.0 ± 7.0	20.2 ± 8.2
Albumin (g/dL)	3.1 ± 0.6	3.2 ± 0.6
LDL (mg/dL)	107.5 ± 36.5	105.5 ± 34.7
HDL (mg/dL)	45.1 ± 11.2	45.2 ± 12.0
Ca (mg/dL)	9.2 ± 0.5	9.3 ± 0.6
P (mg/dL)	3.3 ± 0.6	3.4 ± 0.6
Magnesium (mg/dL)	2.0 ± 0.3	2.0 ± 0.2
ALP (U/L)	86.0 (39.0–178.0)	81.0 (50.0–239.0)
PTH (pg/mL)	56.0 (13.6–172.0)	59.00 (40.0–232.0)
Hemoglobin (g/dL)	13.0 ± 1.8	12.6 ± 1.0
MPV (fl)	6.8 ± 0.9	7.0 ± 1.6
ESR (mm/h)	23.3 ± 13.6	22.8 ± 11.7
CRP (mg/dL)	1.0 (0.1–8.7)	1.3 (0.1–9.4)
Ceruloplasmin (U/L)	887.8 ± 89.5	728.7 ± 102.8*

Parametric data are expressed as mean ± SD; nonparametric data are expressed as median (minimum-maximum)

BMI: body mass index; AST: aspartate transaminase; ALT: alanine transaminase; LDL: low density lipoprotein; HDL: High density lipoprotein; Ca: Calcium; P: phosphorous; ALP: Alkaline phosphatase; PTH: Parathyroid hormone; MPV: mean platelet volume, ESR: erythrocyte sedimentation rate; CRP: C-reactive protein. $p < 0.01$

was found to be higher in geriatric patients with osteoporosis³⁵). In this study we evaluated inflammatory parameters and CP levels in subjects without chronic inflammatory disease. Only geriatric patients with and without osteoporosis were included, with serum CP levels found to be higher in geriatric patients with osteoporosis than in healthy subjects. However, other inflammatory parameters, specifically CRP and ESR, did not differ between the study groups. Surprisingly, serum CP levels above 830.15 U/L exhibited 85.7% sensitivity and specificity in the diagnosis of osteoporosis.

In summary, this study demonstrated that serum CP level is elevated in osteoporosis independently of other inflammatory parameters, and thus may be considered in use as a screening test for osteoporosis. Limitations of the study included a cross-sectional study design and a relatively small sample size. Therefore, future experimental studies are needed to clarify our results.

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