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Original Article

The Erythrocyte Catalase Enzyme Activity in Iranian Osteoporotic Women

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Abstract

Osteoporosis is a disease of high prevalence with increased bone loss. It has potential public health threat and that the health authorities had taken several measures for its control. In recent years, considerable attention has been given to physiopathology of osteoporosis. Oxidative stress has been proved to be involved in bone resorption. Oxidative stress occurs when balance between oxidants and biochemical antioxidants is disrupted because of excess reactive oxygen species. Thus, we measured catalase activity, as one of the marker of stress oxidative, in 138 women. Participants were selected by inclusion and exclusion criteria from those who were referred to Jamie Clinic in Tehran for BMD evaluation. Catalase antioxidant activity was 262.01±44.70 k/gHb in the group of subjects with osteoporosis in comparison with the group of healthy subjects, 273.77±46.92 k/gHb. The results show that catalase activity in patients with bone deficiency was less than in the control group, though it was not significant. This difference was more between control and patients group with more acute disease (T score -1.7) than patients group with milder disease (T score<-1).The results show that it may be useful to monitor osteoporosis in the more expanded sample size to obtain more definition results.

Keywords: Catalase activity; Iranian women; Osteoporosis; Oxidative stress.
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1. Introduction

Osteoporosis is significant changes in bone turnover; bone formation decrease whilst bone resorption increase or remains the same,

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resulting is net bone loss [1]. It is potential public health threat and has to be several measures for its control. Bone fractures are a major cause of burden associated with osteoporosis in terms of mortality, disability, and costs. The common causes of this disease include malnutrition, immobilization, calcium deficiency and many metabolic disorders like diabetes mellitus and hyperparathyroidism [2].

Oxygen can be converted to highly reactive oxygen containing free radicals such as peroxide, singlet oxygen, hydrogen peroxide, and the hydroxyl and hydroperoxyl radicals. Approximately, 5% of cellular oxygen is not used in the production of ATP, is reduced to reactive free radicals [3]. Reactive oxygen species (ROS) are produced continually in body. There are some antioxidants that change the oxygen free radicals to some compounds that they aren't too harmful. Some of them are superoxid dismutase (SOD), catalase and glutation peroxidase enzymes [4].

ROS are generated from many exogenous sources such as toxic agents and chemicals. ROS provoke oxidative stress and compromise the oxidant system of living organisms through lipid peroxidation of membranes, protein cross linkage, mitochondrial damage, and DNA damage.

Recent *in vitro* studies or animal models showed that oxidative stress has an important impact on osteoclast differentiation and function. ROS and antioxidant system might be involved in the pathogenesis of bone loss. In a study is demonestreted that oxidative stress markers are important indicators for bone loss in postmenopausal woman [5]. It is investigated that oxidative stress induced in differentiated cells by a number of different oxidative stimuli and oxidative stress may contribute to the pathology of a number of bone diseases, such as osteoporosis [1].

Over the past several years, great advances have been made in the simplification of reliable diagnostic tests for evaluating certain oxidative stress biological markers, such as the measurement of LPO, GPx, SOD, catalase, total antioxidant status, DNA damage, antioxidant vitamins (A, C, and E), and minerals (zinc, selenium), among others [6]. These stress biomarkers have been surveyed in different tissue for assessing age related disease such as osteoporosis. Therefore, the use of biomarkers to identify patients with oxidative stress may be helpful in managing osteoporosis [7].

The goal of this survey was to assesss activites of catalase, an antioxidant enzyme, in Iranian women with osteoprosis and comparing to the control group to emphasize on correlation between catalase and osteoprosis.

2. Materials and methods

2.1. Procedure

In this study, subjects were screened among a total of approximately 1000 women that referred to bone mineral densitometry division of Jami Clinic in Tehran (Iran). The main exclusion criteria that could interact with interpretation of the results were secondary osteoporosis, diseases caused by oxidative stress, malnutrition, hormone replacement therapy, use of antioxidant vitamins and anti-resorptive drugs, calcium and vitamin D. Accordingly, 186 women were selected of which, 140 women enrolled in the study. The participants were divided into three groups according to the values suggested by the WHO division for Tscore and osteoporosis. The control group were normal in femur and spine and had T score>-1, the total patients [(mild osteopenia+severe osteopenia +osteoporosis)] had T score<-1, in this manner women with femur T score <-1.7, were considered as severe osteopenia +. The project was approved by Ethics Committee of Tehran university of Medical Sciences. The questionnaire included demographic variables (self reported age, body mass index (BMI), history of diseases, nutritional status, smoking habit, functional status and disabilities; self

Table 1. Measurement of catalase activity.

Pipette successively into the cuvette	blank	Sample	Concentration in assay mixture
(1) Phosphate buffer	1.00 ml	-	Phosphate 50 mmol/l
sample (heamolysate)	2.00 ml	2.00 ml	volume fraction 0.67
(2) H_2O_2 solution	-	1.00 ml	H_2O_2 10 mmol/l
Start the reaction by addition of H_2O_2.			
Initial absorbance should be *ca*. A=0.5			
Mix well with a plastic paddle; follow the decrease in absorbance with a recorder for *ca*. 30 s.			

reported fractures and use of medicines. The questionnaire was performed by a trained interviewer. T scores of the femoral neck and lumbar spine of participants was measured, using dual energy X-ray absorptimetry (QDR4500R, Holcic, Acclaim R. series). On the day of the bone densitometry underwent a fasting blood withdrawal in 10 ml heparinized tubes.

2.2. Determination of catalase activity

The chemicals and reagents were purchased from the Merck (Darmstadt, Germany) and Fluka (Steinheim, Germany) companies and all solutions were prepared with distilled water. The pH of solutions was measured by a model 713-pH meter (Metrohm, Herisau, Switzerland). Catalase activity was determined according to Aebi method [8]. Catalase activity was determined spectrophotometrically at wavelength 240 nm. In this method, generally:

1. Phosphate buffer (50 mmol/1; pH 7); a: Dissolve 7.806 g NaH_2PO_4. $2H_2O$ in water and makeup to 1000 ml, b: Dissolve 7.099 g NaH_2PO_4 in water and make up to 1000 ml. Mix the solutions a and b in the proportion 1:1(v/v). The pH of solutions was measured by a model 713-pH meter.

2. Hydrogen peroxide (30 mmol/1) is prepared (dilute 0.34 ml 30% hydrogen peroxide with phosphate buffer to 100 ml).

3. Erythrocytes are washed 3 times with isotonic NaCl

4. A stock haemolysate containing *ca*. 5 g Hb/100 ml by the addition of 4 parts by volume of water.

5. 1:500 dilution of this concentrated haemolysate with phosphate buffer immediately was prepared

6. Haemolyze 0.1 or 0.02 ml of blood in 250 or 50 ml water

7. Haemolysate samples should be analyzed within 5-10 min. of dilution at room temperature

8. The reduction rate of H_2O_2 was followed at 240 nm according to Table 1.

2.3. Statistical analysis

Statistical analysis was performed using SPSS. The data are expressed as the mean±SD or percentage. Descriptive statistics were conducted on all the variables to evaluate range, the variance, frequencies and normality of the resulting data. Demographic and clinical variables were compared by the X^2 test. Correlation analysis was carried out by means of the Spearman test. Analysis of covariance was performed to compare femoral T score as well as catalase activity among the groups, with age and BMD as covariates. Statistical significance was defined as $p<0.05$.

3. Results

Two out of 140 subjects; enrolled in bone mineral densitometry were excluded from the study because of the inadequate data, so total number of subjects was 138. In the present study, the T scores of both lumbar spine (L1-L4) and femoral neck were measured in all participants. Three groups were compared according to the values suggested by the WHO Division for T score

Table 2. Comparison of erythrocytes catalase activity, age, and BMI among control and patient groups.

Groups	Control (Tscore>1)	Total patients (Tscore<-1)	Severe osteopenia+ osteoporosis
	n=54	n=55	(Tscore<-1.7) n=38
Age (year)	55.80±12.38	48.17±11.16	58.13±13.12
BMI (kg/m^2)	28.25±5.02	25.80±3.64	25.36±3.43
Catalase activity (k/gHb)	273.77±46.92	262.01±44.70	258.26±40.03

and osteoporosis. The control group were normal in femur and spine and had T score>-1(n=54, 37.2%), the total patients [(mild osteopenia+severe osteopenia+osteoporosis)] had T score<-1, (n=55, 40.6%), women with femur T score <-1.7, were considered as severe osteopenia+osteoporosis (n=38, 29.6%). A number of 29 patients of total participants entered neither patient group; because of femoral T score>-1, nor control because of spinal T score<-1.

The erythrocyte catalase activity was compared between controls and the patients. Moreover, differences between age, BMI, and disease were observed (Table 2). BMI was associated with femur mineral density (r=+ 0.405, p<0.01), while age was inversely associated with femur mineral density (r=- 0.377, p<0.01).

4. Discussion

Catalase antioxidant activity was 262.01±44.70 k/gHb in the group of subjects with osteoporosis in comparison with the group of healthy subjects, 273.77±46.92 k/gHb. The results show that catalase activity in patients with bone deficiency was less than in the control group, though it was not significant (p=0.60), and it may be useful to monitor osteoporosis and catalase in more expanded sample size for more definition results. This difference was more between control and patients group with more acute disease (T score-1.7, p=0.36) than patients group with milder disease (T score<-1, p=0.68). Femoral neck BMD, T score, showed a positive and significant correlation with erythrocyte activity of catalase in all participants.

Some of disorders that have been attributed to ROS-induced cell dysfunction include 1: Cardiac stunning and arrhythmia; 2: Skeletal muscle injury; 3: Neurological conditions e.g., neuronal damage in Parkinson`s disease; 4: Neurotoxicity; 5: Alzheimer's disease; 6: Diabetes, apoptosis of T lymphocytes, and gastric mucosal injury; and 7: Hypertension [9]. Recently, reactive oxygen species (ROS) are considered to be responsible for the aging process and osteoporosis [10].

Oxidative stress regulates cellular functions in multiple pathological conditions, including bone formation by osteoblastic cells [11]. Oxidative stress is an independent risk factor for osteoporosis [6]. The results of a study indicated that increased osteoclastic activity and decreased osteoblastic activity may be associated with an imbalance between oxidant and antioxidant status in postmenopausal osteoporosis [12]. Osteoblast can produce antioxidant such as glutathione peroxidase to protect against ROS, whilst osteoclast - generated superoxide contributes to bone degradation. Biological markers or biomarkers are fundamental in determination of different diseases. Biomarkers are usually divided to three categories: Biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility [13].

One of the enzymes involved in antioxidant defense, and is known as a biomarker of oxidative stress is catalase. Catalase is one of the most potent catalysts known. It is present in aerobic cells. Catalase converts hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular

oxygen. Hydrogen peroxide elicits a broad spectrum of cellular response. Hydrogen peroxide at high concentrations is deleterious to cells and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids. Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to the cell. Catalase activity in many diseases causes oxidative stress in cells and tissues and has been assessed. For instance, catalase activity in diabetic dental pulps was significantly enhanced in comparison with that of control pulps [14]. Also, in pancreatic disease, catalase activity was higher than control [15]. Oxidative stress is increased in atherosclerotic lesions and might play an important role in plaque progression and calcification. Catalase expression was reduced in pericalcific regions [16]. Catalase activity was decreased in patients with cervical intraepithelial neoplasia and squamous cell carcinoma of the cervix [17].

Therefore, we focused on catalase activity as an effect biomarker of oxidative stress. Biomarkers of effects reflect early biomedical modifications. Catalase activity was less in osteoporetic patients than in control.

As mentioned earlier, to identify patients with oxidative stress are helpful in managing osteoporosis. The present study suggests that antioxidants seem to influence on rate of this disease. Meanwhile, in our previous study, an association between the plasma activities of SOD, vitamin C, E and BMD was observed [18-21]. In another study, it was found that carotenoids which may have beneficial skeletal effects are lower in women with osteoporosis [22], and certain antioxidants were shown epidemiologically to counteract risk of this disease [23]. Therefore, like other disease related to oxygen-derived free radicals [24], antioxidant component should be considered in management and reducing the risk of osteoporosis. For instance, vitamins C and E may reduce the risk of osteoporosis in postmenopausal women with calcium therapy.

In summary, the aim of our study was to evaluate correlation between catalase activity and osteoporosis in women. Catalase activity was less in patients than in control. In conclusion, our findings indicate that oxidants agents could influence on rate of this disease in our population.

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