Determination of plasma gluthatione reductase enzyme activity in osteoporotic women

[1]Sadeghi N., [1]Oveisi M.R., [*2]Jannat B., [1]Hajimahmoodi M., [3]Jamshidi A.R., [1]Sajadian Z.

[1]Department of Drug and Food Contro,l Faculty of Pharmacy, Medical Sciences/University of Tehran,. [2]Food and Drug Deputy, Ministry of Health and Medical Education, [3]School of Medicine, Medical Sciences / University of Tehran, Tehran, Iran

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ABSTRACT

Background: Osteoporosis is a disease of high prevalence with increased bone loss. Free radicals have been proved to be involved in bone resorption. Glutathione reductase (GR) plays an essential role in cell defense against reactive oxygen metabolites by sustaining the reduced status of an important antioxidant, glutathione. In the present study GR activity of plasma as an antioxidant enzyme in relation to Bone Mineral Density (BMD) was investigated.

Material and Method: GR activity was measured spectrophotometrically at 339 nm in 138 women. Participants were selected by inclusion and exclusion criteria from those who were referred to Jamie Clinic in Tehran for BMD evaluation.

Results: Plasma activity of GR (Mean± SD) was: 64.44 ± 37.68 U/L in the control group, 75.49 ± 54.84 U/L in the total patients [(mild Osteopenia + severe Osteopenia and Osteoporosis) (Tscore<-1)] and 80.48 ± 61.91 U/L in the patients with severe Osteopenia and Osteoporosis respectively (Tscore <-1.7).

Conclusion: The results show that plasma activity of GR in patients with bone deficiency was higher than the control group. The difference was more pronounced between controls and the patients with severe Osteopenia and Osteoporosis, than those differences between controls and the total patients. Femur Tscore adjustment with age and BMI showed significant negative correlation with the plasma activity of GR in all subjects.

Keywords: Antioxidant, Glutathione reductase, Plasma, Osteoporosis.

INTRODUCTION

Osteoporosis is a disease of high prevalence with increased bone loss, substantial morbidity and mortality (1, 2), and is common in Iran. It is one of the most critical age-related disorders for postmenopausal women (3). Postmenopausal reduction in Bone Mineral Density (BMD) appears to be associated with excessive osteoblastic activity (4). Gender is one of the risk factors and about 80% of those affected by osteoporosis are women. Ethnicity is another factor and the BMD of Asian women is 5-10% lower than those of African, American or Latino women (5). Other risk factors are age, body type (skeleton type), smoking, alcohol use, lack of exercise, and others (5).

The most common treatments are calcium and vitamin D intake, hormone therapy, the use of bisphosphonates, calcitonin, parathyroid hormone and selective estrogen-receptor modulators(5).

Reactive oxygen species (ROS) are produced by various environmental agents (6) and their formations in cells result in formation of free radicals in metabolic processes (7). These harmful species cause damages to many molecules such as lipids, proteins, nucleic acids and are considered to be responsible for the aging process and a number of pathological conditions such as atherosclerosis, carcinogenesis and osteoporosis (7-8) These harmful effects are controlled by antioxidant defense system of the cells of which the most important free radical chain breaking molecule in various tissues of the body is glutathione (7). Furthermore, enzymes such as superoxid dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase are necessary to remove free radicals and to stabilize the cells (7).

Glutathione reductase (GR) belongs to a family of flavin-containing pyridine nucleotide-disulphide oxidoreductases and the major function of these homologous dimeric proteins are to catalyze the conversion of the oxidized glutathione (GSSG) to the reduced glutathione (GSH) using NADPH as a coenzyme. Accordingly, GR plays a key role in antioxidant

Correspondence: Janatbhr@sina.tums.ac.ir

capacity of the cells through maintaining high ratio of GSH/GSSG (9).

The aim of this study was to measure the activity of GR of the plasma as an antioxidant enzyme in Iranian osteoporotic women in comparison to the healthy ones.

MATERIALS AND METHODS

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 antiresorptive drugs. Accordingly, 186 women were selected of which, 140 women enrolled in the study. The participants were divided into three groups: the control group (Tscore≥-1) of 54 women (39.1%), the total patients [(mild Osteopenia + severe Osteopenia and Osteoporosis) (Tscore<-1)] of 55 women (39.9%) and group with femur Tscore <-1.7, of 38 (women) considered as severe Osteopenia and Osteoporosis. The project was approved by Ethics Committee of Medical Sciences / Tehran University.

The questionnaire included demographic variables (self reported age, body mass index; BMI, history of diseases, nutritional status, smoking habit, functional status and disabilities, self reported fractures and use of medicines). The questionnaire was performed by a trained interviewer. All subjects were on free diet.

Tscores of the femoral neck and lumbar spine of participates were measured, using dual energy x – ray absorptimetry (QDR4500^{R} , Holcic , Acclaim $^{R.}$ series)

The subjects on the day of the bone densitometry, underwent a fasting blood withdrawal in 10 ml heparinized tubes, and after centrifugation, the plasma was distributed into special vials. The vials were temporarily stored in liquid nitrogen and then were analysed.

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).

GR activity was determined spectro-photometrically at 339 nm (10). In this method, generally, 2.4 mL of Imidazole as buffer (0.23 mol/L, PH 6.9, 25C°), 0.1 mL of EDTA (8.25 mmol/L), 0.05 mL of GSSG (114 mmol/L) and 0.1 mL of the sample (plasma) were mixed thoroughly. Five min later, 0.1 mL of NADPH (12 mmol/L) was added, mixed thoroughly, and the absorbance was recorded immediately (less than 5 seconds) with a spectrophotometer (UV visible spectrophotometer, GBC Cintra 40, Victoria, Australia).

The values of $\Delta A/\Delta t$ were determined to be < 0.300/min at 339 nm using the following formula:

$b=F\times\Delta A/\Delta t$ U/L

$F=4.37\times10$ at 339 nm

Statistical analysis was performed using SPSS. The data are expressed as the mean ± SD or as 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 Tscore as well as plasma activity of GR among the groups, with age and BMD as covariates. Statistical significance was defined as $p<0.05$.

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 Tscores 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 Tscore and osteoporosis. The control group with 59 women, were normal in femur and spine and had tscore>-1, the total patients of 55 women [(mild Osteopenia + severe Osteopenia and Osteoporosis) (Tscore<-1)] and group of 38 women with femur Tscore <-1.7, were considered as severe Osteopenia and Osteoporosis.

Plasma GR activity were compared between controls and the patients. No differences was found in the number of diseases, drugs and functional activities between these groups, but the differences were significant when age and BMI were take into consideration ($P<0.05$).

GR activity and femur Tscores were entered into multivariate models as continuous data and then adjusted for age and BMI. None of the interactive factors (age, BMI) had statistically significant associations with the plasma GR activity (thought r values were negative for both).

BMI was associated with femur mineral density ($r = + 0.405$, $p<0.01$), while age was inversely associated with femur mineral density ($r= - 0.377$, $p<0.01$).

It is worth of saying that there were lower plasma GR activities in the smokers than non smokers. Smoking habit was not associated with femur

Table1. Correlation between plasma activity of GR and T–score values.

Group	Femoral neck T–score	n	P	Pearson correlation(r)
Severe Patient	≤ -1.7	38	0.030	- 0.367
Total Patient	≤ -1	55	0.014	- 0.352
Control	>- 1	59	0.703	- 0.059
All participants	-3.9 to 3.6	138	0.398	- 0.085

Tscore but femur mineral density was lower in the smokers compared to the non smokers.

Plasma activity of GR (Mean ± SD) was 64.44 ± 37.68 U/L in the control group, 75.49 ± 54.84 U/L in the total patients with Osteopenia (Tscore<-1) and 80.48 ± 61.91 U/L in the patients with severe Osteopenia and Osteoporosis (Tscore<-1.7).

By adjustment for BMI and age, Tscore was directly examined with the plasma activity of GR (Table1).

DISCUSSION

In this study, GR activity of the plasma among Iranian osteoporotic women showed that: values of GR were higher in the patients than in the control, though differences were not significant. However, differences were higher between the controls and patients with severe disease (Tscore <-1.7). In addition Tscore of femur adjusted with age and BMI showed negative significant correlation with plasma activity of GR in total patients

After adjustment of plasma activity of GR for BMI and age, no significant relation was observed between the plasma activity of GR and femur Tscore in the total participants and the control group, but it was significant for the total patients and severe Osteopenia patients (Table1).

Some investigations have indicated that osteoporosis is associated with biochemical markers of oxidative stress, such as urinary excretion of isoprostanes and reduction of plasma antioxidants (6,11,12). Oxidative stress by itself and its effects on the regulatory cytokines such as tumor necrosis factor and interlukins are involved in osteoporosis. The reactive oxygen species (ROS) are neutalized by the antioxidant system in the body. Osteoblasts produce antioxidants such as glutathione peroxidase to protect against ROS (13). Lipid peroxidation is one of the most harmful effects of ROS, of which malonedialdehyde (MDA) is the end product. Serum levels of MDA increase in bone loss (13-14). A recent report shows that the blood and saliva total antioxidant power of 22 osteoporotic subjects were significantly lower than those of 22 healthy controls, however the blood and saliva thiobarbituric acid- reactive substances (as a marker of lipid peroxidation) 0f osteoprotic subject were significantly higher than those of healthy controls (14). Increased in superxide production by osteoclasts displayed by increased levels of MDA in the serum might results in decreased activities of antioxidant enzymes such as superoxide dismutase and Glutathione peroxidase (13)

Research on age-related changes in glutathione and glutathione reductase–related enzymes have shown that GR and other antioxidant activities are increased in the brains of both aged male and female compared to the young male or female rat brains (15). Another study on antioxidant enzymes in synovial fluid of the patients with primary and secondary osteoarthritis have shown that GR and other antioxidant synovial activities were higher in the patients than in the controls and difference was higher between the controls and the secondary osteoarthritis patients(16).

Several studies have also shown that antioxidant enzymes are increased by oxidative stress and exercise. However, the increase in antioxidant defenses might not be physiologically proportionate to the needs created by the increase in pro oxidant events and thus might affect the requirements for dietary antioxidants (17).

On the other hand, in another report, by investigation of more than 10,000 women between ages of 50 and 80 years, it has been found that total plasma antioxidant enzymes such as glutathione peroxidase and superoxid dismutase in the osteoporotic women were not lower than the non-osteoporotic women (18).

Cellular- molecular happenings in osteoporosis with balance upsetting between osteoblasts and osteoclasts activities cause increase in production of free radicals in bone cells and intracellular space. Thus bone formation decreases due to the increase in bone loss because of inhibitory effects of free radicals on osteoblastic differentiation.

In current study, significant association between the plasma activity of GR and BMD was not observed; though mean plasma activity of GR in patients was higher than those of the control group. It seems that more extensive study with larger sample size might supply definite results about this association.

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