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Role of oxidative stress and inflammatory markers in osteoporosis

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Abstract
Osteoporosis is a major health issue in post-menopausal females, however, it may occur before menopause. In middle aged women, osteoporosis directly relates to low bone mineral density (BMD) that consequently increases the risk of bone fracture. Several factors are involved in osteoporosis, however, the relationship of osteoporosis with oxidative stress and inflammatory response still needs to be investigated. In the current study, decreased values of different antioxidants displayed that they are necessary in maintaining bone homeostasis. A variety of all the oxidative stress parameters observed in this study, can be very effective to curing the disease.

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Introduction

Osteoporosis (OP) is a chronic multifactorial disease which is characterized by low bone density and breakdown of bone tissue. The cause of OP is the variation between the development of osteoblasts (bone forming cells) and osteoclasts (bone breaking cells) (Bird and McAuley, 2019). The activity of osteoblasts is inhibited by enhancing the activity of osteoclasts (Hung et al., 2021). Osteoporosis is associated with the risk of bone fractures due to weakness of bones. Back pain, loss of height and fractures are the most common symptoms in osteoporosis (Gorter et al., 2021). In some cases, it occurs without clear-cut symptoms, because loss of bone occurs gradually over many years, so it is called as "silent disease" (Szamatowicz, 2016). It mostly occurs in females and mature population of greater than 50 years (Zheng et al., 2018). A number of reports have shown that osteoporosis may give rise to many other diseases, and major being the osteonecrosis (Matthews et al., 2022). The risk factors that cause low BMD (bone mineral density) are diastolic blood pressure, skeletal fragility, more tea consumption, medicines such as glucocorticoids and anticonvulsants, low calcium and vitamin D intake, falls, alcohol consumption and smoking (Hyun et al., 2020).

Free radicals such as nitric oxide (NO) and hydroxyl radical (OH^-) comprising single unpaired electrons, can effectively destroy bone tissue and may lead to oxidative stress (OS) (Vladana et al., 2017). Molecular oxygen reacts with radicals and make free radicals that cause a direct damage to our biological molecules (Phaniendra et al., 2015). These radical species can be of two types, ROS (reactive oxygen species) and RNS (reactive nitrogen species). Antioxidants are the part of defense mechanism that activate to neutralize the mechanism of ROS by enhancing the immune defense mechanism (Liu et al., 2018). The major types of antioxidants include catalase (CAT), vitamins (A, C, D and E), superoxide

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dismutase (SOD), and glutathione (GSH). The levels of antioxidants are low in patients associated with different types of abnormalities such as osteoporosis, because antioxidants help in bone protection and healing process.

Malondialdehyde (MDA) is a marker of oxidative stress that is immediately released as a final product of lipid peroxidation and is usually high in patients of OP because of increased activity of osteoclasts (Gaweł et al., 2004). Nitric oxide increases in patients of inflammation-induced OP (IMO) due to increased values of blood pressure (BP) with low BMD (Jacomini et al., 2016). It has an inverse relation with antioxidant vitamin D (fat soluble vitamin). It is believed that physical exercise may help improve the NO production by endothelial nitric oxide synthase (eNOS) in patients (Tsukiyama et al., 2017). It is obvious that oxidative stress plays a major role in causing OP, because oxidative stress overcomes the defensive mechanism of antioxidants (Sheweita and Khoshhal, 2007). It has been shown that malnutrition, low physical activity, and high doses of drugs are considered as the main cause of osteoporosis in premenopausal women (Genest et al., 2021). In post-menopausal osteoporosis, high doses of bisphosphonates are prescribed, but these are not prescribed in premenopausal osteoporosis, because of the reason that high doses can affect the lifestyle of patients (Genest et al., 2021). ROS activate the osteoclast activity, and hence, cause low BMD, which ultimately leads to osteoporosis (Hung et al., 2021). However, information on the risk of fracture reduction is little in the literature.

Clinical trials provide evidence that healthy diet with supplements of vitamin D and calcium can help in cure of osteoporotic patients (Liu et al., 2020). In a recent study, OP treatment has proved that intake of healthy diet with vitamin D and calcium supplements can help in prevention of the disease (Liu et al., 2020). The main objective of the current study was to determine the role of vitamin D in females of age 30-35, and to ascertain if low levels of vitamin D and calcium significantly relate to low BMD.

Materials and Methods

Source of data

A total of 90 females of age 30-35 were included in this study that were screened at Jinnah Hospital Lahore, Pakistan. This research work was properly permitted by the "Research and Ethics Committee" at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan. A blood sample measuring 5.0 mL was taken from the vein of each patient. The blood was further processed for the identification of various oxidative markers, antioxidants, blood analysis and various inflammatory markers. The samples were collected from the females suffering from osteoporosis. The blood samples were centrifuged at 1789 *g* for 10 min and the serum was separated and collected into EDTA tubes for further analysis.

Biochemical analysis

Different biomarkers such as SOD, MDA, CAT, Vit-C, Vit-E and Vit-A were analyzed using the spectrophotometric method in control and osteoporotic patients. The levels of SOD were measured following Maqbool et al. (2019). The concentration of MDA was measured as illustrated by Ohkawa et al. (1979). CAT was measured according to Maqbool et al. (2019). The levels of reduced glutathione and glutathione peroxidase were measured by the method described elsewhere (Moron et al., 1979). The concentration of GSH was estimated using the method described elsewhere (Maqbool et al., 2019). Vitamin C and Vitamin E were estimated by the methods illustrated elsewhere (Mohammad et al., 1991). Furthermore, nitric oxide concentration was measured by the colorimetric Griess assay (Hunter et al., 2013). Vitamin D levels were estimated using an ELISA kit (Maqbool et al., 2019).

Statistical analysis

Statistical analysis of data for all attributes was performed using the SPSS (Statistical Package for the Social Sciences). One-way ANOVA (Analysis of variance) was worked out for each variable. Pearson's correlation coefficient was used to determine the correlations between different values of the variables of osteoporotic patients. P-value was calculated using one-way ANOVA.

Results

It was found that the mean value of MDA (1.2±0.956 nmol/mL vs. 0.65±.065 nmol/mL) and NO (65.25±12.25 µmol/L vs 16.5±2.25 µmol/L) were significantly higher in the patients with osteoporosis as compared to those in the control individuals correspondingly (**Figure 1**). On the other hand, the levels of GSH, GPx and GRx were significantly lower in the osteoporotic females (1.36±.0325 µmol/L, 6.3±2.35 µmol/mL and 1.99±0.025 µmol/mL) than those in the control group (10.25±3.29 µmol/L, 7.6±2.059 µmol/mL and 1.23±.058 µmol/mL), respectively (**Figure 2**). Decreased levels of vit. A, E, C, and D were

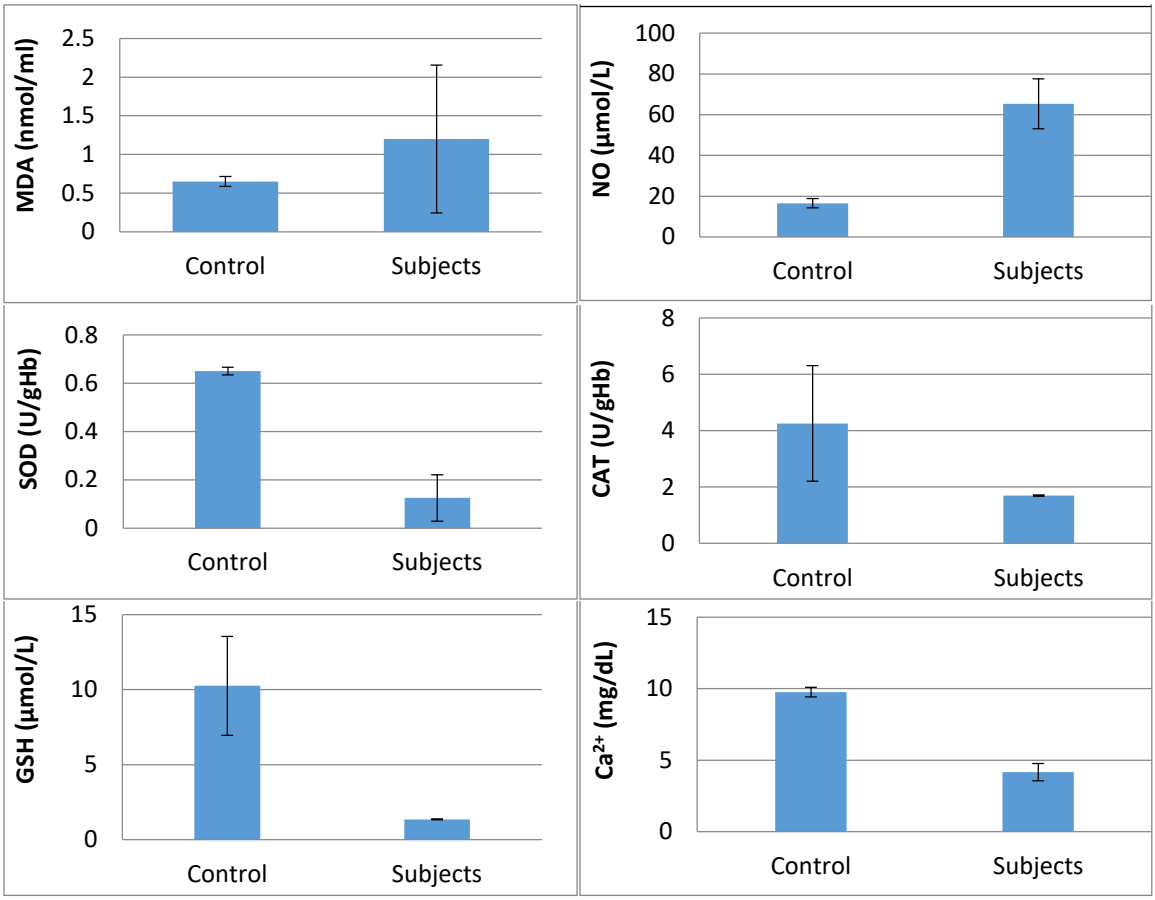

Figure 1. Levels/activities of different oxidative stress parameters in osteoporotic female patients and normal individuals.

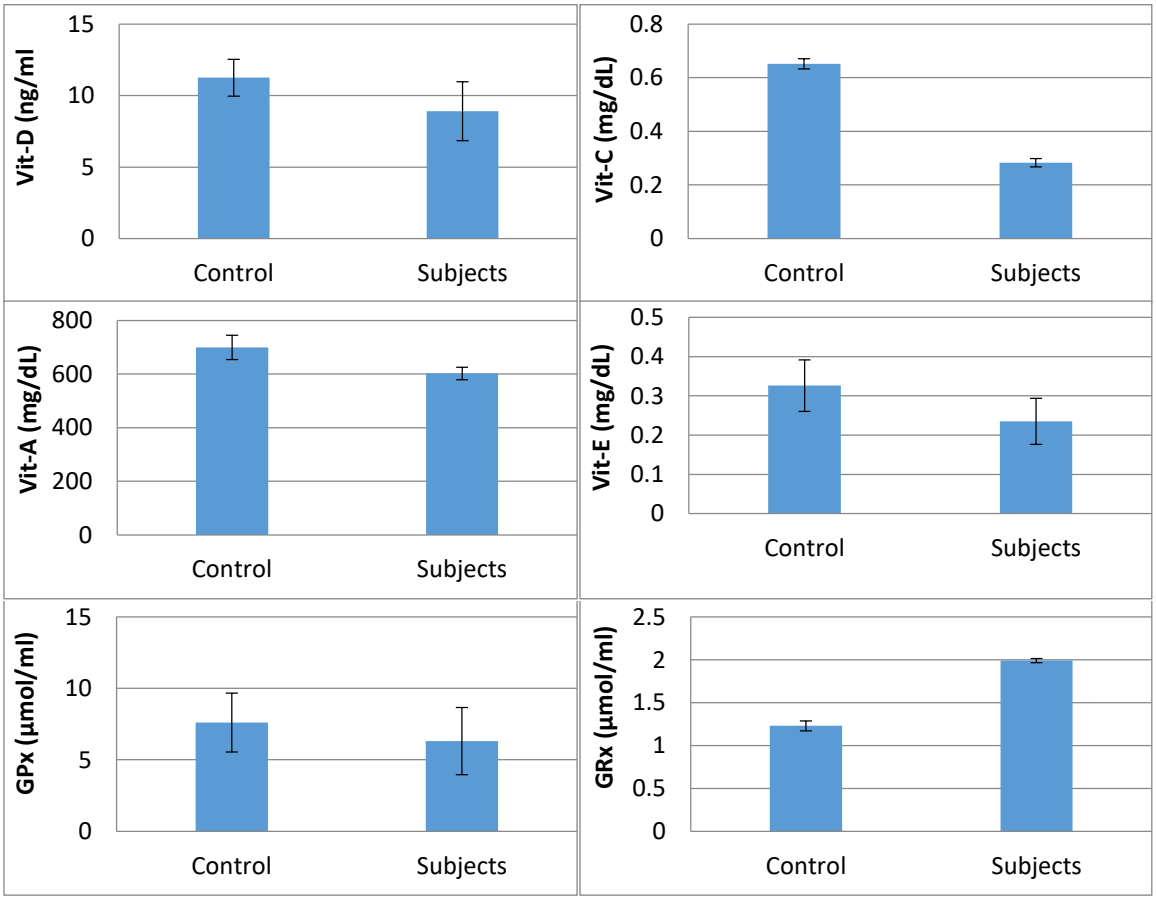

Figure 2. Levels/activities of different calcium and vitamin parameters in osteoprostic female patients and normal individuals.

measured in the osteoporotic females (602.26±23.26 mg/dL, 0.235±.059 mg/dL, 0.283±.0156 mg/dL, and 8.9±2.056 ng/mL) in comparison with those in the control individuals (699.35±45.29 mg/dL, 0.326±.0659 mg/dL, 0.652±.019 mg/dL and 11.25±1.29 ng/mL) (**Figure 2**). Moreover, the reduced activities of CAT (1.7±.0259 U/gHb vs. 4.26±2.056 U/gHb) and SOD (0.125±.0956 U/gHb vs. 0.65±.0156 U/gHb) (**Figure 1**) were recorded in the osteoporotic females as compared to those in the control individuals, respectively.

Discussion

Osteoporosis (OP) is a serious health issue worldwide, which affects almost every community. Treatment for OP is available, but there are various risk factors that are highly involved. Vitamin D plays an important role in maintaining body structure and health. To maintain health status, 20 µg daily intake of vitamin D is required. Vitamin D helps in absorption of calcium in bone and decreases the risk of causing OP, and bone turnover (Liu et al., 2020). 1,25-dihydroxyvitamin D (1,25-OH-2D) is an active metabolite of vitamin D that increases absorption of calcium in the gut, and hence, the chances of bone mineralization increases (Lips and van Schoor, 2011). Vitamin D deficiency can also cause secondary hyperparathyroidism due to lack of exposure to sunlight (Sizar et al., 2022).

In the current study, the level of vitamin D in the OP patients was lower (8.9±2.056 ng/mL) compared to the control (11.25±1.29 ng/mL). Vitamin D deficiency occurs when the level of serum 25 hydroxyvitamin D decreases below 50 nmol/L in the body cells (Brincat et al., 2015). Mostly, doctors prescribe vitamin D and calcium supplements to make serum 25 hydroxyvitamin D levels above or equal to 75 nmol/L (Sizar et al., 2022). These supplementations help in recovery and less bone loss in elderly women, but all supplements are not equally effective. Vitamin D2 is less effective than vitamin D3, but one alpha-cholecalciferol is more effective than D3, because of its synthetic derivation (Tripkovic et al., 2012). The National Osteoporosis Guideline Group (NOGG) suggested that the daily intake of 1000 mg of calcium, 800 U of vitamin D, and 1 g/kg body weight of protein can be helpful in the prevention of OP (Compston et al., 2009).

Accumulation of free radicals are a major cause of peroxidation of lipids that damages polyunsaturated fatty acids, essential for normal growth (Domazetovic et al., 2017). A report indicated that this process is completed in three stages including initiation, propagation, and termination (Catalá., 2006). This damage causes the production of toxic metabolites like MDA and 4-hydroxynonenal (HNE) that have harmful effect on cell membrane of erythrocytes (Liu et al., 2018). Their effect can be prevented by antioxidants like glutathione peroxidase (Ayala et al., 2014). In our research work, we used MDA as a biomarker of oxidative stress (OS) that occurs in the cell. We found that serum MDA was higher in patients associated with osteoporosis (1.2±0.956 nmol/mL) in comparison with that in the control (0.65±.065 nmol/mL) before treatment. Increased osteoclasts activity in many bone disorders is the main cause of increased production of free radicals that consequently lead to increased levels of serum MDA in osteoporotic patients (Hung et al., 2021).

Nitric oxide (NO) is a diatomic free radical as well as a signaling molecule. It affects the function, regulation and maturation of osteoclasts and osteoblasts (Bergmann et al., 2011). It has a significant importance in research and medicine because of its presence in almost all cell types (intracellularly and extracellularly) (Csonka et al., 2015). In our study, we found a significant increase in the levels of NO in patients of OP (65.25±12.25 µmol/L) in comparison with the control (16.5±2.25 µmol/L). NO is a major cause of inflammation in OP, because it highly affects the bone cells by decreasing BMD (Armour et al.,1999; Hof and Ralston, 2001). An evidence shows that low levels of NO are necessary for normal functioning of osteoclasts (Anastasio etal., 2020). NO affects vitamin D regulation, and so has an indirect effect on bone homeostasis, thereby causing OP (Hao et al., 2019).

SOD is an antioxidant, which acts as an endogenous defense system for the treatment of inflammatory diseases (Maqbool et al., 2019). It is used to balance the levels of ROS during disease condition in the body of aerobic organisms (Birben et al., 2012). Our data indicated that the level of SOD was lower in the patients (0.125±.0956 U/gHb) than that in the controls (0.65±.0156 U/gHb), probably due to activation of defense system before supplementation.

GSH is also an endogenous antioxidant which works as a part of intracellular defense system against OS (Sharma et al., 2012). Its levels were significantly lower in the patients (1.36±.0325 µmol/L) than those in the controls (10.25±3.29 µmol/L). GPx helps in the oxidation of GSH in the presence of GRx. GPx is an antioxidant, which acts as a first line of defense, and is used to degrade and neutralize the effect of hydrogen peroxide (Bonaccorsi et al., 2018). The concentration of GPx was found to be low in the patients (6.3±2.35 µmol/mL) relative to that in the control (7.6±2.059 µmol/mL). While the levels of GRx were found to be high in the patients (1.99±0.025 µmol/mL) with respect to those of the control group (1.23±.058 µmol/mL). CAT is also an antioxidant, which is present in all living cells. It has the ability to use

oxygen (Ighodaro and Akinloye, 2018). CAT has the ability to detoxify hydrogen peroxide. In our current study, the activities of CAT in the patients associated with osteoporosis (1.7±.0259 U/gHb) were lower relative to those in the controls (4.26±2.056 U/gHb). Deficiency of CAT is responsible for accumulation of hydrogen peroxide, which leads to oxidative damage on bones (Yang et al., 2022).

In our study, different vitamins that function as antioxidants like vitamin A, C and E were found to be in low concentrations in the OP patients with respect to those in the control group. Vitamin A has the ability to boost up immune system and its deficiency can cause serious bone problems including OP, but large amount is also dangerous for BMD (Ahmadieh and Arabi, 2011). Vitamin C is an antioxidant required for collagen formation and development of bone (Aghajanian et al., 2015). Vitamin E is also an antioxidant that is believed to help in loss of calcium from bones by increasing bone density.

Conclusion

Oxidative stress is a significant cause of many diseases including bone loss during OP. MDA and NO act as biomarkers because they increase a number of osteoclasts, which cause production of free radicals like hydrogen peroxide. Antioxidants were studied because of their effect to neutralize the disease. The decreased values of antioxidants such as CAT, SOD and GPx are considered as the activation of the first line of defense of immune system. Low levels of vitamins in the body reflect low BMD. All parameters discussed in this study can help cure OP, because they have the ability of increasing bone density.

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