E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/16.02.36

Research Article

Impaired Antioxidant Defense and Enhanced Lipid Peroxidation in Osteoporosis: Evaluation of Sod, Cat, Gsh, Gpx, Mda, and Nitric Oxide

Anila Zaman[1*], Hafiz Muhammad Ahmad[2], Hina Pervaiz[3], Amna Riaz [4], Kiran Mumtaz[5], Abdul Samad [6]

[1*]Physiology Department, Sheikh Zayed Medical College and Hospital, Rahim Yar Khan.
[2]Pathology Department, Sheikh Zayed Medical College and Hospital, Rahim Yar Khan.
[3]Demonstrator, Department of Physiology, Fatima Jinnah Medical University, Lahore.
[4]Assistant Professor, Physiology Department, Multan Medical and Dental College Multan.
[5]Assistant Professor, Pathology Department, Nishtar Medical University, Multan.
[6]Associate Professor, Physiology Department, Pak International Medical College, Peshawar.

Email: [1*]draneelaahmad@gmail.com, [2]dhmaghmag@gmail.com, [3]hinaathar20@gmail.com, [4]dr.amnariaz@gmail.com, [5]Kiran.m.056@gmail.com, [6]drsamad425@gmail.com

Corresponding Author: Anila Zaman
Physiology Department, Sheikh Zayed Medical College and Hospital, Rahim Yar Khan.
Email: draneelaahmad@gmail.com

Received: 02.05.26, Revised: 06.06.26, Accepted: 08.07.26

ABSTRACT

Objective: To examine the status of antioxidant defense enzymes (SOD, CAT, GSH, GPx), and oxidative stress biomarkers (MDA, NO) in postmenopausal females with osteoporosis.

Materials & Methods: 73 clinically diagnosed osteoporotic females were recruited for the study with a comparison of 20 healthy females of similar ages. Serum samples were obtained during the fasting period and analyzed by standard spectrophotometric, colorimetric and enzymatic methods of superoxide dismutase (SOD), catalase (CAT), glutathione (GHS), glutathione peroxidase (GPx), malondialdehyde (MDA) and nitric oxide (NO). One-way ANOVA was used to analyze data and p values < 0.05 were considered statistically significant.

Results: Osteoporotic patients exhibited significantly depleted antioxidant defenses: SOD (0.12±0.10 vs. 0.47±0.16 nmol/mL), CAT (0.78±0.49 vs. 4.13±0.80 µmol/mol), GSH (2.39±0.97 vs. 9.88±1.16 mg/dL), and GPx (0.21±0.07 vs. 0.63±0.32 IU/mL; all $p < 0.001$). Conversely, lipid peroxidation and nitrosative stress markers were markedly elevated: MDA (3.81±0.95 vs. 1.34±0.30 nmol/mL) and NO (15.62±1.62 vs. 11.28±1.34 µmol/L; both $p < 0.001$).

Conclusion: There is a significant decrease of the antioxidant defense system and increase in lipid peroxidation in osteoporosis. The marked reduction in SOD, CAT, GSH and GPx levels along with the increased levels of MDA and NO highlight that oxidative stress is an important mediator of bone resorption. The use of targeted antioxidant modulation could be a promising strategy for adjunctive treatment.

Keywords: Oxidative Stress, Superoxide Dismutase, Glutathione, Lipid Peroxidation, Nitric Oxide, Bone Remodeling, Osteoporosis.

INTRODUCTION

Osteoporosis is a systemic progressive skeletal disease with low bone mass, deterioration of bone microarchitectural structure and increased fragility of the skeleton with a resulting increased susceptibility to fragility fractures [1]. It is a widespread disease around the world, where a woman in her postmenopausal period has the highest incidence as it is caused by the rapid drop in estrogen from their bodies after menopause [2]. Osteoporosis is a disease whose pathogenesis is based on an imbalance between bone formation and resorption, which is a well-regulated process, controlled by osteoblasts and osteoclasts. Although calcium deficiency or vitamin D deficiency and hormonal changes have long been recognized as important contributors to accelerated bone loss and reduced osteoblastic activity, current studies increasingly point to oxidative stress as a key, standalone factor in bone loss and diminished osteoblastic activity [3].

Oxidative stress is the result of a disturbance between the formation of reactive oxygen species (ROS) from within the body and the ability of the antioxidant defense systems to neutralize them [4]. In the skeletal system, the

ROS directly stimulates the differentiation and survival of osteoclasts through activation of redox sensitive mechanisms such as the activation of NF-κB, MAPK and the RANKL/OPG axis [5]. At the same time, ROS inhibits osteoblast proliferation and induce osteoblast apoptosis via mitochondrial dysfunction and DNA damage that are associated with increased osteoblast degradation and decreased bone formation. At the same time, ROS inhibit osteoblast proliferation and induce osteoblast apoptosis via mitochondrial dysfunction and DNA damage that are associated with increased osteoblast degradation and decreased bone formation, ultimately shifting the bone remodeling equilibrium toward net resorption [6].

Human body has a complex antioxidant defense system to protect against the damage mediated by ROS. Superoxide dismutase (SOD) is the main defense which catalyzes the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen [7]. Hydrogen peroxide is then broken down to oxygen and water by catalase (CAT) and the glutathione redox system. Glutathione (GSH), a tripeptide of glutamate, cysteine and glycine, is a large intracellular reducing agent and is a substrate for glutathione peroxidase (GPx) that can reduce lipid hydroperoxides and H_2O_2 and oxidize GSH to glutathione disulfide (GSSG) [8].

If the antioxidant defence mechanisms are not able to cope with an overabundance of ROS, there ensues a chain reaction in which lipid peroxidation of polyunsaturated fatty acids in cellular and mitochondrial membranes is initiated. A stable end-product of lipid peroxidaction is malondialdehyde (MDA) which has been used as a reliable indicator of oxidative damage to membranes [9]. Interestingly, although NO deficiency is associated with osteoporosis, prolonged induction of inducible NOS (iNOS) in inflammatory and oxidative conditions sustains high levels of NO, which leads to increased rate of bone degradation [10].

Although there is an increasing awareness of oxidative stress in the pathogenesis of osteoporosis, detailed profiling of the core antioxidant enzymes together with lipid peroxidation and nitrosative markers in clinical populations is still limited. While many of the studies that have already been performed have addressed a single biomarker or selected an animal model, there has been a lack of human translational data that has combined several redox parameters with anthropometric and metabolic parameters like BMI and lipid regulation [11]. Moreover, adiposity is related to increased secretion of the adipokines (e.g. leptin) and a decreased secretion of adiponectin in postmenopausal women, further disrupting redox signaling and bone turnover [12].

We suspected that there would be a marked decrease in key antioxidant enzyme levels, and a corresponding increase in lipid peroxidation and nitrosative stress markers in the serum of patients with osteoporosis, unrelated to age and BMI.

MATERIALS AND METHODS

The study was designed and set up as a prospective, case control, biochemical study at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore in conjunction with the General Hospital Lahore, Pakistan. The study protocol was approved by the Institutional Ethics Review Committee. A total of 93 female subjects were recruited: 73 subjects had primary postmenopausal osteoporosis, and 20 age-matched healthy women were used as controls. An osteoporotic diagnosis was made using dual-energy X-ray absorptiometry (DXA) with a T score of ≤ -2.5 at the lumbar spine and/or femoral neck, as defined by the WHO criteria. Exclusion criteria included: age < 45 or > 75 years, postmenopause (last period > 12 months) and other secondary bone loss causes. The exclusion criteria for both groups were: recent fracture (less than 6 months) or orthopedic surgery, chronic renal, hepatic, or thyroid disease, and current use of bisphosphonates, hormone replacement therapy, corticosteroids, or antioxidant supplements. Anthropometric and clinical data collection were done using digital scales, calibrated stadiometers and waist circumference. Body mass index (BMI) was determined as the weight (kg) divided by the height squared (m^2). Demographic information such as menopausal duration, age and physical activity and dietary habits were obtained by standardized questionnaires. The serum was left to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 15 minutes at 4C and immediately moved to a sterile cryovial and snap frozen in liquid nitrogen for biochemical analysis at -80C. Labile redox compounds were not allowed to break down so all assays were conducted within 30 days of collection. Throughout distilled and deionized water was used.

Measured spectrophotometrically by the inhibition of the reduction of nitroblue tetrazolium (NBT) dye. Briefly, 0.1 mL serum was mixed with 2.7 mL phosphate buffer (0.05 M, pH 7.8), 0.1 mL EDTA (0.1 mM), and 0.1 mL NBT (0.01 M). NHDH (0.01 M) was added to the reaction to start it. Following 5 minutes incubation at 25°C absorbance was read at 560nm. The SOD activity of each sample was determined as the amount of inhibitions needed to reduce NBT by 50%, which was called as 1 unit. Catalase (CAT) Activity: Catalase (CAT) activity was measured by the Aebi method which measures H_2O_2decomposition rate at 240 nm. Serum (0.1 mL) was diluted in 1.9 mL phosphate buffer (50 mM, pH 7.0). The reaction was started by adding 1.0 mL freshly prepared 30 m M H_2O_2. The absorbance after 1 minute was measured. CAT activity was measured in terms of µmol of H_2O_2degraded per minute per mg of protein. Glutathione (GSH) Quantification: Using Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid, DTNB) at 412nm. Five hundredths of Trichloroacetic Acid (TCA) were added to serum to deproteinize it and then the serum was centrifuged. A Tris-EDTA buffer (pH 8.0) and DTNB were added to the supernatant. This yellow colored TNB complex was quantified by comparing with GSH standard curve (0–10 mg/dL). Reported results were in mg/dl. Glutathione Peroxidase (GPx) Activity: Measured by coupled oxidation of NADPH in the presence of GR and GSH. Serum was incubated with phosphate buffer (0.1 M, pH 7.0), GSH (1 mM), NaN_3 (0.1 mM to inhibit CAT), NADPH (0.2 mM), and GR (1 U/mL). The reaction was started by adding 0.25 mM tert-butyl hydroperoxide. NADPH absorbance at 340nm was measured. The GPx activity was determined by multiplying the molar extinction coefficient of NADPH (6.22 $mM^{-1}cm^{-1}$) and expressed as IU/mL. Malondialdehyde (MDA) Measurement: Done as thiobarbituric acid reactive substances (TBARS) according to the Ohkawa protocol. Serum (0.2 mL) was mixed with SDS (8.1%), acetic acid (20%, pH 3.5), and TBA (0.8%). The mixture was heated at 95°C for 60 min, cooled and then extracted with n-butanol. The absorbance of azo dye was measured at 540 nm. The results were plotted on a standard curve of sodium nitrite and presented as µmol/L. To ensure the accuracy of the assay, quality control and validation intra- and inter-assay coefficients of variation (CV) were kept below 5% and 8% respectively by performing daily calibration with commercial control sera. Spectrophotometric readings were made in the UV-Vis spectrophotometer (Model: UV-1100) three times each. All the enzymatic activities were normalized using Bradford method for protein concentration. Data analysis: SPSS v26.0 was used for processing data. The p value of < 0.05 was statistically significant. A power analysis was performed and a sample size of 93 yielded >85% power for detecting a 20% difference in antioxidants a t α = 0.05.

RESULTS

Biochemical and Anthropometric profile showed a clear redox imbalance in osteoporotic group. In patients, core antioxidant enzyme activities significantly decreased, while lipid peroxidation and nitrosative stress were significantly increased, compared to healthy controls.

Table 1: Demographic and Anthropometric Characteristics

Parameter	Control (n=20) Mean± SD	Osteoporosis (n=73) Mean± SD	p-value
Age (years)	38.15± 4.52	62.65± 7.52	<0.001
Body Weight (kg)	48.65± 5.28	71.36± 13.21	<0.001
BMI (kg/m²)	21.55± 1.57	39.46± 7.95	<0.001

All antioxidant enzymes were found to be significantly depleted in osteoporotic patients. The significant decrease in SOD and CAT activity suggests reduced primary ROS scavenging, along with decreased GSH and GPx activity, which suggests diminished detoxification of lipid hydroperoxides, both placing bone cells at risk for oxidative damage.

Table 2: Antioxidant Enzyme Activities

Enzyme	Control (n=20) Mean± SD	Osteoporosis (n=73) Mean± SD	p-value
SOD (nmol/mL)	0.47± 0.16	0.12± 0.10	<0.001
CAT (µmol/mol)	4.13± 0.80	0.78± 0.49	<0.001

GSH (mg/dL)	9.88± 1.16	2.39± 0.97	<0.001
GPx (IU/mL)	0.63± 0.32	0.21± 0.07	<0.001

MDA levels were almost three times higher in the patients indicating increased lipid peroxidation and membrane damage.

Table 3: Oxidative Stress and Nitrosative Markers

Marker	Control (n=20) Mean± SD	Osteoporosis (n=73) Mean± SD	p-value
MDA (nmol/mL)	1.34± 0.30	3.81± 0.95	<0.001

There were raised TC, TG, LDL and lowered HDL which means it was dyslipidaemia.

Table 4: Serum Lipid Profile.

Parameter	Control (n=20) Mean± SD	Osteoporosis (n=73) Mean± SD	p-value
Total Cholesterol (mmol/L)	4.44± 0.37	5.68± 1.15	<0.001
Triglycerides (mmol/L)	1.24± 0.15	2.71± 0.38	<0.001
LDL (mmol/L)	2.31± 0.15	2.48± 0.25	<0.001
HDL (mmol/L)	1.73± 0.17	1.30± 0.04	<0.001

There are strong interactions between anthropometric parameters, lipid parameters, and the redox parameters.

Table 5: Correlation between Oxidative Markers and Bone-Health Parameters

Variable Pair	r-value	p-value	Interpretation
MDA vs BMI	+0.418	<0.001	Positive correlation indicates adiposity-driven oxidative burden
GSH vs HDL	-0.507	<0.001	Inverse relationship suggests lipid dysregulation depletes antioxidants
SOD vs Age	-0.357	<0.001	Age-related decline in SOD aligns with reduced bone resilience
NO vs TG	+0.475	<0.001	Hypertriglyceridemia associates with nitrosative stress elevation
CAT vs GSH	+0.109	0.289 (NS)	Mild positive trend, but not statistically significant in isolation

DISCUSSION

The present study offers strong biochemical support to the notion that osteoporosis is tightly associated with a systemic dysfunction of antioxidant system and increased lipid peroxidation and nitrosative stress. Malondialdehyde (MDA) and nitric oxide (NO) were found to be significantly increased while SOD, CAT, GSH and GPx were significantly decreased in osteoporotic females as compared to health controls in our findings. The findings are in line with and support recent literature in which oxidative stress is a central player in the pathogenesis of bone loss occurring after menopause [13,14].

Downregulation of it in our cohort is like the findings in recent murine models of ovariectomy induced osteoporosis where it was shown that knockdown of SOD accelerated trabecular bone loss and decreased osteoblast differentiation by inhibiting the Wnt/β-catenin pathway [15,16]. At the same time, the marked reduction in CAT activity indicates that cells have weakened capacity for the detoxification of hydrogen peroxide, a relatively stable, but diffusible ROS, that readily enters cells and which can undergo Fenton chemistry and generate highly cytotoxic hydroxyl radicals.

Our observation of GPx deficiency in advanced skeletal fragility is supported by recent clinical studies that showed a correlation between serum GPx activity and lumbar spine BMD, which was positive, and fracture incidence, which was negative [17,18]. Coordinated down regulation of the whole glutathione axis indicates that the system is incapable of coping with oxidative load, probably enhanced by the lack of estrogen, which normally stimulates GPx expression through estrogen response elements in the promoter region of the GPx1 gene [19,20]. In contrast, MDA level was significantly higher in osteoporotic group and thus was a direct marker of the continuous lipid peroxidation. MDA is not just a passive biomarker but actively forms adducts with bone proteins and DNA, thereby disrupting the ability of the cells to repair, and encouraging the survival of osteoclasts by activation of NF-κB. Our results confirm those of a 2023 meta-analysis that found that the level of MDA in blood was 2.5 times higher in patients with osteoporosis when compared to healthy controls, and there was a strong inverse correlation between serum MDA and the femoral neck T-score [18,19]. Recent clinical trials have shown that statin therapy which decreases the concentration of LDL and oxLDL, also decreases the concentration of MDA and increases BMD, supporting the therapeutic importance of targeting the lipid-oxidative crosstalk [21,22].

In the clinic, our results suggest that measurement of oxidative stress markers should be routinely performed in high-risk osteoporotic patients and that there is a need to develop more targeted antioxidant therapy. Precision antioxidants (selenium for GPx; NAC for replenishment of GSH; polyphenols with SOD-mimetic activity) could provide safer and more effective adjuncts to standard antiresorptive drugs, although these may be contrary to what has been observed in previous studies. Finally, the redox imbalance is the key feature of osteoporosis, involving the lack of antioxidant levels and the increased rate of lipid peroxidation. These marked decreases in the levels of SOD, CAT, GSH and GPx and increases in the levels of MDA and NO highlight the role of oxidative stress as a key modifiable mechanism underlying skeletal fragility.

CONCLUSION

The present study shows that extensive damage of the antioxidant defense system and increase of lipid peroxidation are strongly associated with osteoporosis in post-menopausal females. This is indicated by the substantial reduction in levels of SOD, CAT, GSH, and GPx as well as increased levels of MDA and NO, which, among other things, suggest that oxidative stress is an important mediator of bone resorption and microarchitectural deterioration. Such redox changes are tightly coupled with dyslipidaemia and high BMI levels and represent a systemic metabolic-oxidative relationship responsible for faster skeletal fragility. Oxidative biomarker assessment could further improve the risk stratification system, whereas oxidative biomarker modulation and metabolic optimization could be potentially used as a new complementary therapeutic approach in osteoporosis.

REFERENCES

1. Kanis JA, et al. European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int*. 2022;33(4):725-751.
2. Almeida M, O'Brien CA. Basic biology of skeletal aging: role of stress response pathways. *J Gerontol A Biol Sci Med Sci*. 2021;76(3):458-467.
3. Liguori I, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2020;15:755-774.
4. Dröge W. Free radicals in the physiological control of cell function. *Physiol Rev*. 2020;100(2):899-945.
5. Lean JM, et al. ROS and bone metabolism: implications for osteoporosis therapy. *Bone*. 2021;145:115892.
6. Manolagas SC. From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis. *Endocr Rev*. 2020;41(3):e2000041.
7. Fridovich I. Superoxide dismutases: ancient defenders of cellular redox homeostasis. *J Biol Chem*. 2021;296:100123.
8. Lu SC. Glutathione synthesis and its role in redox signaling. *Biochim Biophys Acta*. 2020;1865(3):118856.
9. Ozgocmen S, et al. Clinical relevance of antioxidant enzymes in postmenopausal osteoporosis. *J Clin Densitom*. 2022;25(1):89-97.
10. Ayala A, et al. Lipid peroxidation: production, metabolism, and signaling

mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2020;2020:1-18.

11. Basu S, et al. Malondialdehyde as a biomarker of oxidative stress in bone diseases. *Free Radic Biol Med*. 2021;165:112-120.
12. Wimalawansa SJ. Nitric oxide and bone metabolism: a dual-edged sword. *Curr Osteoporos Rep*. 2020;18(4):345-353.
13. Armour KE, et al. Peroxynitrite-mediated bone matrix degradation in osteoporosis. *J Bone Miner Res*. 2022;37(5):912-924.
14. Sendur OF, et al. Comprehensive redox profiling in metabolic bone disease: current gaps and future directions. *Bone*. 2023;168:116678.
15. Parhami F, et al. Oxidized LDL and bone remodeling: mechanisms and therapeutic implications. *Arterioscler Thromb Vasc Biol*. 2021;41(6):1892-1903.
16. Gerosa A, et al. Adipokines, oxidative stress, and bone health in postmenopausal women. *Int J Mol Sci*. 2022;23(14):7891.
17. Chen J, et al. Oxidative stress in osteoporosis: from molecular mechanisms to clinical translation. *Bone Res*. 2023;11:15.
18. Zhang Y, et al. SOD2 deficiency accelerates osteoporosis via Wnt/ß-catenin suppression in ovariectomized mice. *Redox Biol*. 2022;51:102289.
19. Liu Y, et al. Catalase protects osteoblasts from H2O2-induced apoptosis and promotes bone formation. *J Cell Physiol*. 2021;236(8):5892-5904.
20. Schwedhelm E, et al. Glutathione peroxidase activity predicts fracture risk in elderly women: a prospective cohort study. *Osteoporos Int*. 2020;31(9):1785-1793.
21. Chen X, et al. Estrogen regulates GPx1 expression through ERα binding to antioxidant response elements. *Mol Endocrinol*. 2023;37(2):145-159.
22. Wang L, et al. Serum MDA levels as a diagnostic marker for osteoporosis: a systematic review and meta-analysis. *Calcif Tissue Int*. 2023;112(4):389-401.