ORIGINAL ARTICLE

Association of Serum Glutathione with Bone Mineral Density in Females with Osteopenia and Osteoporosis

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Abstract

Background: Low bone mineral density is a hallmark of osteoporosis. Changes in the levels of reactive oxygen species and the body's natural antioxidant system lead to oxidative stress, which, by modulating the activity of osteoblasts and osteoclasts, can lead to bone resorption and, ultimately, fracture and associated morbidities.

Objective: To find the association of serum glutathione with bone mineral density in females with osteopenia and osteoporosis in Lahore, Pakistan.

Methods: A cross-sectional study was conducted in Shalamar Hospital, Lahore, from September 2022 to August 2023. After a written informed consent form, 100 females above 42 years of age were selected as study participants. Demographic characteristics, including height, weight, and blood pressure, were recorded. The bone mineral density of the left calcaneum was determined with Sonost 3000, and 0.5ml of intravenous blood sample was taken.

Results: Average age of study participants was 52±8 years with the majority of females between 50-58 years of age. This study found a statistically significant association of serum glutathione with bone mineral density ($p<0.05$) and serum glutathione levels greater than 4.2ng/dl possess a 41.4% protective effect in the development of osteoporosis in the adjusted model.

Conclusion: The current study's results indicate the need for glutathione, a non-enzymatic antioxidant, to prevent or at least halt osteoporosis. The present study may also suggest the potential role of glutathione as a biochemical marker for the diagnosis of oxidative stress in bone.

Keywords: *Oxidative stress, bone mineral density, osteoporosis, antioxidant, glutathione.*

INTRODUCTION

Osteoporosis (OP) is characterized by a decline in bone mineral density (BMD) and microarchitectural deterioration of bone tissue [1]. OP remains a global burden of aging with a prevalence of 19.7% in the middle to elderly population and a higher incidence in developing countries [2]. In Asia, osteoporosis affects 10-30% of females above 40 years of age [3]. In Pakistan, the present risk of OP is 9.9 million, out of which 7.2 million are females, and it is predicted to rise to 12.1 million by the year 2050 [4]. It is estimated that almost 500-1000 people/ 0.1 million suffer from OP-related fractures in their 50's [5], which means a fracture is happening every 3 seconds to someone, somewhere in the world [6].

Dual-energy X-ray absorptiometry (DXA) is the gold standard for diagnosis of OP, fracture risk assessment, and patient surveillance but due to its cost constraints and ionization hazards, it's not readily available everywhere. Among other methods, a Quantitative ultrasound scan (QUS) is an alternative option with almost equal significance, when BMD is assessed on the left calcaneum or phalanx of the left thumb [7, 8]. According to the World Health Organization, a BMD value of ≤-2.5SD is a cut-off value for the diagnosis of OP, and a value between -1 to -2.5 SD is considered for osteopenia, while levels of BMD above -1 SD are said to be normal [9].

OP is categorized into primary and secondary OP, irrespective of the type, oxidative stress (OS) is the hallmark of the disease [10]. OS is the imbalance between the production of reactive oxygen species and antioxidants, that results in inhibition of osteoblast activity, stimulation of osteoclastic bone resorption, their growth and differentiation along with inhibition of bone mineralization [11]. Glutathione(GSH) is the most potent non-enzymatic antioxidant that promotes osteogenesis and prevents RANK-L-associated osteoclastic activity [12, 13]. A recent study reported that GSH is essential for neutralizing reactive oxygen species and providing a pro-osteoblastic environment for bone formation.

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Due to the higher incidence of osteoporosis in females and its associated morbidity, screening of this vulnerable group at an early stage can reduce the disease burden. This study will explore the role of GSH in normal and osteoporotic bone. This study aims to measure the serum glutathione levels in elderly females with normal and low bone mineral density and find its association with BMD. This may help in the early diagnosis and management of females who are at risk of osteoporosis, particularly in setup with no access to DXA scans or even QUS.

METHODOLOGY

After approval from the institutional review board and ethics committee (REF: SMDC-IRB/AL/31/2022), a cross-sectional study was conducted in collaboration with the orthopedics department of Shalamar Hospital Lahore, Pakistan. The study lasted from September 2022 to August 2023.

By taking the prevalence of osteoporosis in females of Pakistan as 12.9% [14]. CI=95% and the margin of error is 6.57%. The calculated sample size was 71; however, we recruited 100 subjects.

A total of 100 females above 42 years of age were selected *via* nonprobability convenient sampling, and females who were known cases of osteoporosis or taking antioxidants, supplements, or any ailment that can predispose to the early development of osteoporosis, such as Diabetes, Renal failure, Liver disease, chemotherapy, any other bone disease, *etc.* were excluded.

After written informed consent, demographic details were noted on a study-designed proforma, and height in meters and weight in kilograms were measured by a wall-mounted stadiometer and weighing scale respectively. This was followed by measurement of BMD of left calcaneum by QUS and reading of T/Z score was noted. 0.5 ml of intravenous blood sample was taken under complete aseptic measures. Blood was allowed to clot at room temperature for 30 minutes and then centrifuged at 3000RPM for 15 minutes, 50µl of serum was separated, aliquots were made and stored frozen at -80°C till further analysis. Serum GSH levels were measured with ELISA kits at the end of data collection.

Data was analyzed with SPSS 26.0. The normality of data was assessed with the Shapiro-Wilk test. Descriptive statistics were expressed as mean±SD or frequency and percentages. The Mann-Whittney U test was applied to check the statistical difference across the two groups for study variables, as the majority of study variables, like age, BMI, and glutathione were not normally distributed). The Association of Glutathione with bone mineral density was assessed with a chi-square test of association followed by binary logistic regression analysis before and after adjusting age and BMI. A p-value of <0.05 was considered significant.

RESULTS

This study included 100 females above 42 years of age. Stratification of age revealed that the majority of females were between 50-58 years of age. Data revealed that the majority of females were postmenopausal, and the average age of menopause reported in our study was 49 years (**Table 1**).

Table 1: Descriptive statistics of the study population.

Variable (n=100)	Mean	SD
Age (years)	52.90	8
Weight (kg)	71.73	13
Height (feet)	5.08	0.4
BMI (kg/m^2)	26	
Blood Pressure		
Systolic (mmHg)	117	5.5
Diastolic (mmHg)	85	10
	n	**%age**
Menopause		
Yes	57	57
No	43	43

SD=standard deviation.

Based on the bone mineral density, the study population was divided into 3 groups, women with normal BMD (T/Z score >-1SD), women with osteopenia (T/Z score -1 to -2.5 SD), women with osteoporosis (T/Z score ≤-2.5SD), study variables were measured, and data was depicted in terms of means and standard deviation. The majority of females had low bone mineral density, but the prevalence of osteopenia was almost equal to osteoporosis (**Fig. 1**). Data revealed no difference in mean values of BMI and height of normal and osteoporotic females, but the age of osteoporotic females was slightly greater than normal females while their weight was comparatively less compared to normal females (**Table 2**). When BMD categories were divided into normal and low bone mineral density (including both osteopenia and osteoporosis), and the Mann-Whittney U test was applied to check the statistical difference across the variables, a significant difference between age,

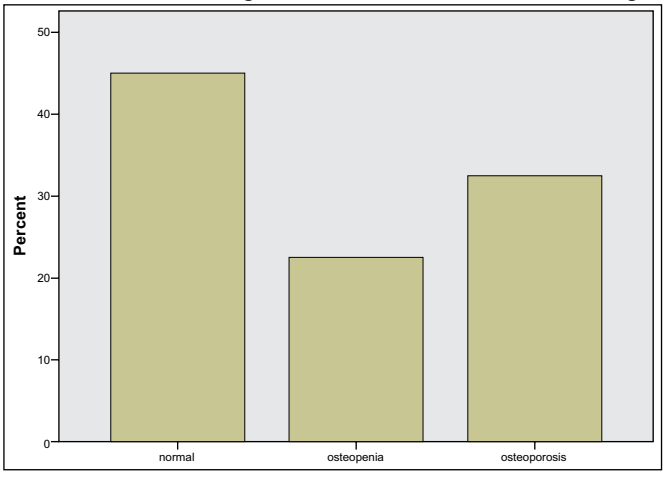

Fig. (1): Frequency and percentage distribution of females with normal and low bone mineral density (osteopenia and osteoporosis). Normal (n=45,), osteopenia (n=22), osteoporosis (n=33).

Table 2: Mean difference across variables, based upon bone mineral density categories.

Variables	BMD Categories	Mean	SD	p-value
Age	normal	49.7	9.5	0.003*
	osteoporosis	50.8	8.9	
Weight	normal	70.3	14	0.002*
	osteoporosis	66.9	9.6	
Height	normal	5.1	0.42	0.187
	osteoporosis	5.1	0.48	
BMI	Normal	28.9	7	0.002*
	osteoporosis	28.3	7.2	
Glutathione	normal	5.7	3.7	0.016*
	osteoporosis	5.4	3.5	

n=100 females (normal, n=45, osteoporosis, n=33, rest of the females were in category of osteopenia, n=22). Mann-Whittney U test applied. *A p-value of <0.05 was considered significant.

Table 3: Association of bone mineral density with glutathione (n=100).

Serum GSH	Normal BMD	Low BMD	Total	p-value
Normal	27 (27)	19(19)	46 (46)	0.006*
Oxidative stress	8 (8)	46 (46)	54 (54)	
Total (n)	35 (35)	65 (65)	100 (100)	

Values are expressed in n (%). GSH= Glutathione, BMD=bone mineral density. The chi-square test of association was applied. *p-value is significant (<0.05).

weight, BMI, and serum glutathione levels of normal and osteoporotic females is observed (p=<0.05) (**Table 2**).

Serum glutathione levels were measured in ng/ml and ≤4.1ng/ml [15] was taken as a cut-off value of oxidative stress. Our results also found a strong association between glutathione levels and bone mineral density categories (p<0.05), depicting that circulating levels of glutathione have a strong connection with changes in bone mineral density and the development of osteoporosis (**Table 3**).

One unit decrease in serum glutathione levels from 4.2ng/dl will lead to 31.5% odds of having osteoporosis with a very strong association (β=1.157, 95% CI= 0.10-0.97), in a non-adjusted model. However, this effect was reduced after adjusting age and BMI, which indicates a strong interplay between these variables. (β=0.534, 95% CI=0.14-0.36) (**Table 4**).

Table 4: Binary Logistic Regression Analysis for odds of having osteoporosis in non-adjusted and adjusted model.

Variables	β	Exp β	CI	p-value
Non-adjusted Model				
Glutathione	1.157	0.315	0.10-0.97	0.046
After Adjusting Age and BMI				
Glutathione (ng/dl)	0.534	0.586	0.14-0.36	0.045
BMI (kg/m^2)	0.514	0.598	0.79-4.51	0.619
Age (years)	0.146	1.157	1.06-1.26	0.001

BMD was a dependent variable. CI=Confidence Interval

DISCUSSION

Our study included 100 females *via* nonprobability convenient sampling. The average age of our study population was 52 years and the majority of females were postmenopausal with an average age of menopause at 49 years. This average age of menopause of our study population was consistent with the studies involving lower to middle socioeconomic status [16, 17]. While some studies reported the average age of menopause at 51 years [18]. This difference is most probably due to differences in the socioeconomic status of the study participants.

Our study also reported that with advancing age, osteoporosis becomes more common, and a decrease in BMD is also strongly associated with an increase in weight and BMI while we couldn't establish any significant association of BMD with height, although, females with osteoporosis were shorter in height. These results can be due to degenerative changes in the spine, attributable to osteoporosis. In the current study, levels greater than 4.2ng/dl significantly reduced the chances of osteoporosis by 68.9% in the unadjusted but 41.4% in the adjusted model, suggesting a potent non-enzymatic molecule in halting the disease phenomenon. These results were reinforced by some previous studies that determined BMD's association with BMI [19] and advancing age [20], but these didn't establish any association with the weight of study participants. A few studies also reported results, opposite to our findings, that BMI is positively correlated with BMD [1, 21]. These differences can be due to the different study selection criteria, as these studies included only postmenopausal females with advanced age, and the factors that affect osteoporosis and BMI were not excluded, while our study included middle to old age females and they were all healthy females with no known comorbidities.

The present study reported the prevalence of low bone mineral density to be 55% while osteoporosis was found in 33% of the study population. This study reported a significant association of GSH with BMD (p<0.05). Previous studies demonstrated that increased enzymatic antioxidant levels like superoxide dismutase, and alpha-tocopherol maintain normal BMD [9]. However, none of the previous studies reported the activity of reduced GSH to changes in bone mineral density. The findings of our study are consistent with previous studies regarding changes in the antioxidant levels correlated with changes in bone mineral density, the only difference is the measurement of non-enzymatic antioxidants (GSH).

CONCLUSION

The current study highlights the significant impact of changes in glutathione levels on BMD, highlighting its potential role in bone health. A strong and significant association between BMD and GSH suggests that serum glutathione measurement in healthy perimenopausal females could be an effective tool for the preclinical screening of osteopenia and osteoporosis. Additionally, the association between BMI and BMD emphasizes the importance of lifestyle modifications, particularly focusing on increasing muscle load rather than fat to enhance shearing stress on bones, thereby improving

bone strength. Adopting a healthy diet rich in natural antioxidants could further strengthen bones and reduce the risk of early-onset osteoporosis.

Future research should explore longitudinal studies to validate the use of glutathione as a predictive biomarker for bone health. Investigations into the molecular mechanisms linking oxidative stress, glutathione levels, and bone metabolism could provide deeper insights. Moreover, interventional studies focusing on dietary, lifestyle, and pharmacological strategies to modulate glutathione levels and their subsequent impact on BMD are warranted.

ETHICS APPROVAL

This study was approved by the Institutional Review Board and Ethics Committee of Shalamar Medical and Dental College, Lahore, Pakistan (REF: SMDC-IRB/AL/31/2022). All procedures performed in studies involving human participants were following the ethical standards of the institutional and/ or national research committee and the Helsinki Declaration.

CONSENT FOR PUBLICATION

Written informed consent was taken from the participants.

AVAILABILITY OF DATA

Data supporting the results of the study can be provided upon request to the corresponding author.

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None.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Declared none.

AUTHORS' CONTRIBUTION

MS: Conception of study design, data recruitment, initial draft

SF: Conception of study design, data analysis, final manuscript

IA: Data analysis, initial draft, final approval

RYK: data recruitment, initial draft, final approval

RBS: Data analysis, Data interpretation, initial draft

RR: Data recruitment, Data interpretation, initial draft

MA: Data recruitment, Data interpretation, initial draft

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