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Original Article

Exploring the effects of homocysteine metabolism in osteoporosis management in Indian adult females

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Abstract: *Objectives:* Women are at a significantly higher risk of osteoporotic fractures, largely due to progressive bone demineralization and impaired bone microarchitecture. Low bone mineral density (BMD) is a common condition in women worldwide. Disrupted homocysteine (Hcy) metabolism has been linked to reduced BMD and increased risk of osteoporotic fractures. Hyperhomocysteinemia (Hhcy) affects osteoblast and osteoclast activity, interferes with collagen cross-linking in the extracellular matrix, and has a detrimental effect on bone health. This study aimed to establish the association between hematological and biochemical parameters and osteoporosis in adult females. *Methods:* We measured Hcy, creatinine, uric acid (UA), vitamin B12, and vitamin D levels. Significantly elevated Hcy (27.322 ± 0.816 vs 10.152 ± 0.381 µmol/L), creatinine (0.670 ± 0.012 vs 0.587 ± 0.011 mg/dL), and UA (5.118 ± 0.083 vs 2.786 ± 0.060 mg/dL) were found in osteoporotic females, while reduced concentrations of vitamin B12 (148.883 ± 2.192 vs 294.14 ± 6.505 pg/mL) and vitamin D (24.98 ± 0.621 vs 33.7 ± 0.652 ng/mL) were observed. *Results:* Hematological parameters were found differentially expressed in osteoporotic females. Elevated Hcy levels, combined with reduced vitamin B12 and vitamin D, were strongly associated with decreased BMD and a higher susceptibility to osteoporotic fractures. Women with increased Hcy levels also had lower T-scores compared to those without Hhcy. *Conclusions:* These findings suggest that Hcy plays a critical role in bone resorption and osteoporotic fractures. Regulating Hcy metabolism may serve as an effective therapeutic strategy for managing bone resorption and osteoporosis. We hypothesize that elevated Hcy levels are closely related to low BMD and an increased risk of osteoporosis.

Keywords: Osteoporosis, BMD, homocysteine, vitamin B12, vitamin D, creatinine, uric acid, DEXA, bone resorption

Introduction

Osteoporosis is a skeletal disorder characterized by progressive bone demineralization and a significant loss of bone mass, which increases fragility and the risk of fractures. Globally, it is a major health concern, with approximately 200 million cases and 8.9 million fractures annually, leading to significant morbidity and economic costs. In India, the prevalence of osteoporosis among females is notably high, ranging from 8% to 62% in various regions. Women are particularly at risk due to factors like early menopause, nutritional deficiencies, and lifestyle choices [1-5].

Recent research has identified elevated homocysteine (Hcy) levels, known as hyperhomocysteinemia (Hhcy), as a potential contributor to bone fragility. Hhcy has been shown to negatively impact on bone health by influencing osteoclast and osteoblast activity and interfering with collagen cross-linking in the bone matrix, leading to reduced bone mineral density (BMD) and a higher risk of osteoporotic fractures. Despite extensive studies, the specific

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role of Hcy in bone metabolism remains incompletely understood [6-11].

The metabolism of Hcy is regulated through two major pathways: the transmethylation pathway and the transsulfuration pathway. In the transmethylation pathway, Hcy is remethylated to methionine, either through a folate/vitamin B12-dependent process, which involves N-5-methyltetrahydrofolate (MTHF), or through a folate/vitamin B12-independent process that utilizes betaine. N-5-methyltetrahydrofolate remethylation occurs in all tissues, while the betaine pathway is primarily confined to the liver. In contrast, the transsulfuration pathway involves the conversion of homocysteine to cystathionine through cystathionine β-synthase (CBS), which is vitamin B6-dependent. Cystathionine is further broken down into cysteine and α-ketobutyrate by cystathionine γ-lyase (CTL). These metabolic processes are essential for maintaining cellular Hcy balance and preventing its accumulation in the body [12, 13].

Figure 1 illustrates the cellular metabolism of Hcy, highlighting the critical enzymes and cofactors involved in its regulation. Disruption in these pathways, such as deficiencies in vitamins B6, B12, or folate, can lead to the accumulation of Hcy, which is associated with various pathological conditions, including cardiovascular diseases, neurodegenerative disorders, and bone diseases like osteoporosis [14, 15].

In this study, we aim to investigate the relationship between Hcy metabolism and osteoporosis in adult Indian females. By analyzing key biochemical markers such as vitamin B12, vitamin D, creatinine, and UA, alongside Hcy levels, we seek to better understand how Hhcy contributes to bone resorption and increased fracture risk. We hypothesize that elevated Hcy is directly associated with lower BMD and a higher risk of osteoporosis in this population.

Materials and methods

Study design

This case-control study has been designed to evaluate the different risk factors associated with osteoporotic fracture including the measurement of Hcy concentration in female patients. Osteoporotic patients were randomly recruited in the study. Significant hematological (Complete Blood Count, CBC) and biochemical parameters (creatinine, UA, vitamin B12, and vitamin D), were measured in adult Indian females suffering from osteoporosis.

Selection of volunteers: 156 female participants age group 40-55 years were recruited randomly from two Hospitals; 1. Jaggottam Ayurveda Panchakarma Centre, and 2. Ashutosh Hospital and Trauma Centre in Uttar Pradesh, India. The enrolled participants were divided into two groups; I) 77 clinically diagnosed Osteoporotic Females (OF) were considered as the case group experiencing osteoporosis from last two years of enrollment, and II) 79 healthy Non-osteoporotic Females (NOF) who never diagnosed osteoporosis were considered as control group. Study participants were screened with the help of pre-listed health history questionnaires for any medical complications such as Diabetes, Asthma, CVD, Depression, Anxiety, and other mental disorders. The objective of the study was clearly explained to the participants, and their written consent was obtained prior recruiting. This study has been approved by the population resource and research center, Institutional Ethics Committee, University of Allahabad, Praygaraj, Uttar Pradesh, India, with the IERB ID: 2022-30DOBC.

Inclusion criteria: I. Age Group: Female participants aged 40 to 55 years were included. This age group was selected as it represents a critical period where the risk of osteoporosis increases significantly, particularly around the perimenopausal and menopausal stages. II. Osteoporosis Diagnosis: Participants must have been clinically diagnosed with osteoporosis at least two years prior to enrollment. This diagnosis was confirmed using the Dual-energy X-ray Absorptiometry (DEXA) scan with a T-score of ≤-2.5 SD, in accordance with the World Health Organization (WHO) criteria for osteoporosis. III. Body Mass Index (BMI): Participants with a BMI between 18.5 and 29.9 were included to focus on non-obese individuals, as extreme BMI values (both underweight and obesity) could potentially confound the relationship between bone density and biochemical parameters. IV. Literacy: Only literate participants were included to ensure that they could independently understand and respond

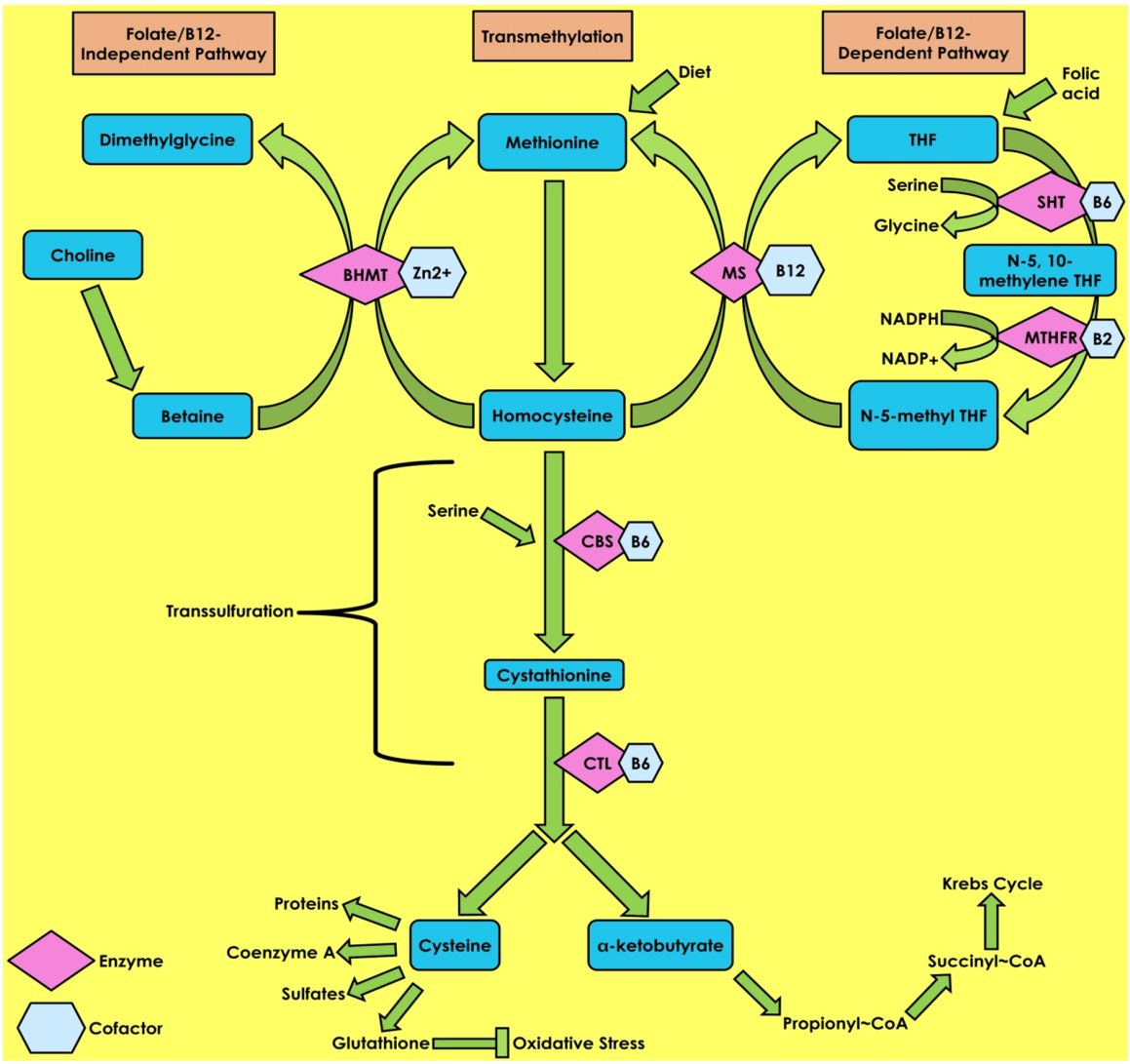

Figure 1. Homocysteine (Hcy) metabolism in the human body. The cellular metabolism of Hcy is properly balanced by two pathways: 1) Transmethylation pathway and 2) Transsulfuration pathway. In remethylation pathway methyl group supplied to Hcy either by N-5-methyltetrahydrofolate (MTHF, folate/vitamin B12 dependent) or by betaine (folate/vitamin B12 independent). N-5-methyltetrahydrofolate remethylation occurs in all tissues whereas betaine remethylation is confined mainly to the hepatic cells. In the transsulfuration pathway, Hcy reacts with serine to form cystathionine catalyzed by vitamin B6 dependent cystathionine β-synthase (CBS). Further, cystathionine is hydrolyzed to form cysteine and α-ketobutyrate by the enzyme γ-cystathionase lyase (CTL).

to the study's questionnaires and give informed consent. V. Willingness to Participate: All participants signed a written informed consent form before enrollment, signifying their voluntary participation in the study. They were also briefed on the study's objectives and methodology prior to participation.

Exclusion criteria: I. Chronic Illnesses and Disorders: Participants with any known chronic illnesses that could affect bone metabolism were excluded. This included but was not limited to: 1) Diabetes mellitus; 2) Cardiovascular diseases (CVD); 3) Renal diseases; 4) Liver diseases; 5) Endocrine disorders; 6) Autoimmune disorders. II. Menopause Status: Females who experienced menopause before the age of 40 (early menopause) or who reported amenorrhea lasting more than 6 months were excluded due to the significant influence of menopause on bone health. III. Pregnancy: Pregnant women were excluded in the study.

Questionnaires: Anthropometric data, medical history, and bone disease including osteoporosis for each volunteer were obtained by the indi-

vidual demographic questionnaires. The enrolled volunteers responded to the questionnaires to obtain information regarding age, sex, marital status, literacy, socioeconomic status, chronic illness, disease duration, medication, and use of any supplement. Supplement intake was not considered in the dietary intake. The validated physical activity scale for the elderly (PASE) was used to assess the physical activity of volunteers [16].

Anthropometric measures

All the enrolled volunteers underwent anthropometric measurement, the body weight in kilograms (kg), and height in centimeters (cm) were measured. Body Mass Index (BMI, kg/m^2) was calculated. According to International Standards (IS), BMI between 18.5 to 24.9 was considered normal; 25 to 29.9 considered overweight; >30 considered obese, class I (30-34.9), class II (35-39.9), and class III (>40) [17].

Diagnosis of osteoporosis

The assessment of existing BMD, evaluating the fracture risk, and focusing on appropriate therapeutic intervention, are the ultimate goals for osteoporosis management. WHO defined the threshold value ≤2.5 SD in T-score of young healthy females for confirming osteoporosis.

Dual-energy X-ray absorptiometry (DEXA): DEXA is a gold standard and widely accepted technique which clinically used to measure BMD at any site (central or peripheral) of the skeleton system and assist in the diagnosis of osteoporosis. DEXA has a major advantage as it exposes nearly 90% less radiation in patients than chest radiographs. DEXA accomplished BMD at the site of bone measurement by passing two beams of light with different energies. The unit of areal bone density measurement is (g/cm^2). DEXA analyzes BMD at the peripheral (distal radius and the calcaneus) is less significant in determining the risk of fractures than spinal and hip DEXA. A low BMD reported by peripheral DEXA is insufficient in the diagnosis of osteoporosis or treatment strategy, however, it may warrant to assessment of spine, hip, and femoral neck BMD. The BMD of all volunteers was measured by DEXA technique and categorized as normal (≥-1 SD), osteopenia (-1 to -2.5 SD), and osteoporosis (≤2.5 SD) based on T-score [18, 19].

Blood sample collection, storage, and processing

Venous blood was collected from both OF and NOF groups at aboard certified pathology by an experienced phlebotomist. EDTA coated anticoagulant vials were used for hematological assessment and plasma separation whereas plain vials for serum separation. All separations (blood cells, plasma, and serum) collected and frozen at -80°C temperature for later use [20].

Biochemical measurement

Quantification of homocysteine: Hcy circulates in the body either in free form or as mixed disulfide (Hcy-Cys) and protein-bound form. To achieve tHcy concentration, all forms (free, disulfide, and protein-bound forms) must be accounted. Highly sensitive and reproducible methods for Hcy estimation included high-performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LCMS), and immunoassays. Plasma tHcy levels were measured in all OF and NOF samples as described earlier with minor modifications [12, 21]. The tHcy quantification was performed on HPLC (Shimadzu, Japan). Hcy standards and chemicals were purchased from Merck (Source: Merck India). The chemicals used in the HPLC technique: 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (ABD-F), Tri-n-butylphosphine, Cysteamine, L-Homocysteine and L-Cysteine. In the experiment, all samples were subjected to the reduction reaction that enabled the SH/thiol group to free. The thiol-specific fluorescent 4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole was labeled to free thiol group. The protein content in the sample was precipitated and collected from the supernatant. Now in the supernatant, free and reduced Hcy were present and labeled with ABDF which was further used to analyze. For HPLC analysis, the precipitated protein pellets were hydrolyzed by the conventional process to remove protein-bound ABDF labeled Hcy. Further, the Hcy amount present in the supernatant and in protein hydrolyzed fractions were collected and quantified. Hcy concentrations were expressed in μmol/L, the concentration between 5-15 μmol/L was considered normal, and >15 μmol/L was considered elevated in the body's fluids.

Hematological assessment/complete blood count: Hematological analysis (CBC) was performed on an automated hematology analyzer (Agappe, India). A list of 21 blood parameters including Leukocytes (WBC count, MID, Lymphocytes, Granulocytes), Erythrocytes (RBC count, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD), and Platelets (PLT count, MPV, PDW, P-LCC, P-LCR) were counted in given volume of fresh blood. According to IS, blood cell count was expressed as WBC count (10^3/μL), RBC count (10^6/μL), HGB (g/dL), and PLT count (10^3/μL) [22].

Quantification of creatinine: Creatinine assessment has a significant role in clinical Biochemistry. Its concentration in serum and urine indicates the efficiency and normal physiology of several organs in body. The quantification of creatinine in all OF and NOF volunteers was done with minor modifications of the method given by [23]. UV-Vis Spectrophotometer was used to standardize and quantify creatinine in serum samples. Its concentration has been expressed in (mg/dL). The widely accepted reference intervals of creatinine in males (0.72-1.18 mg/dL) and in females (0.55-1.02 mg/dL) [24].

Quantification of uric acid: UA is a nitrogenous waste product formed by the metabolism of nucleotides (Adenine and Guanine). Increased UA in Plasma, Serum, and Urine correlates the metabolic complications. It may induce several disorders including bone disease, stone formation, and kidney infection. The important risk factor for UA crystallization and stone formation is a low urine pH (<5.5), happen due to impaired urinary UA excretion. The spectrophotometric method has been employed to quantify UA in OF and NOF volunteers with minor modifications of standardized method. The reference range of UA concentration in males is greater than females (2.5-7.0 mg/dL in males and 1.5-6.0 mg/dL females) [25, 26].

Quantification of vitamin B12: A list of methods including Radioimmunoassay (RIA), Enzyme-linked immunosorbent assay (ELISA), UV-Vis spectroscopy, Mass spectroscopy, Raman spectroscopy, Microbiological assay, HPLC, Capillary electrophoresis, etc. usually employed to measure the vitamin B12. Apart from other methodologies HPLC (Shimadzu, Japan) is commonly used to measure vitamin B12. Plasma vitamin B12 levels were measured in all OF and NOF samples as described earlier with minor modifications [27, 28]. Vitamin B12 standard and other chemicals were purchased from Merck, India. Vitamin B12 concentrations are expressed in pg/mL.

Quantification of vitamin D: In clinical practice, Chemiluminescence immunoassays (CLIA) method is routinely employed to measure vitamin D in human serum. The total 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites were measured in all OF and NOF volunteers. In methodology, 25-hydroxy vitamin D is dissociated from vitamin D binding protein (DBP) and binds to the specific antibody, followed by vitamin D isoluminol tracer added. All unbound materials are removed with washing. Further, the reagents are mixed to begin the chemiluminescent phenomena. The photomultiplier unit detects the light signals as relative light units and the measurement of 25-hydroxyvitamin D concentration is inversely proportional. The unit used to express the vitamin D concentration is (ng/mL) [29].

Statistical analysis

The comparisons were made between the socio-demographic, clinical, and laboratory measurements using appropriate parametric tests in study groups. The statistical data processing was carried out by using Microsoft Excel 2019 and GraphPad Prism version 10 program. Pearson's correlation coefficient was also calculated among the groups. The result has been presented as the mean and standard error of the mean (SEM). The statistical significance of the difference was verified using the t-test at 95% confidence and *p*-value at ($P<0.05$).

Results

Anthropometric comparison

The anthropometric (Age, Height, Weight, and BMI) comparison was made between the study groups, i.e., OF and NOF. Age in (years), height in (centimeters), weight in (kilograms) were measured and BMI (kg/m^2) was calculated individually. Osteoporotic history was also recorded. As shown in (**Table 1**), age, and body weight of females suffering from osteoporosis were observed slightly higher than healthy females.

Table 1. Anthropometric and bone mineral density measurement in osteoporotic females and non-osteoporotic females

Anthropometric measures	Osteoporotic females (cases) n=77		Non-osteoporotic females (controls) n=79	
	Mean	SEM	Mean	SEM
Age (years)	49.66	± 0.960	48.65	± 0.828
Height (m)	1.53	± 0.008	1.58	± 0.009
Weight (kg)	69.20	± 1.142	63.65	± 1.046
BMI	29.71	± 0.567	25.62	± 0.478
BMD (T-score)	-2.77	± 0.021	-0.72	± 0.023

All values are expressed as Mean and Standard Error of Mean (SEM).

Table 2. Assessment of hematological parameters in osteoporotic females and non-osteoporotic females

Complete blood count (CBC) measurement	Osteoporotic females (cases) n=77		Non-osteoporotic females (controls) n=79	
	Mean	SEM	Mean	SEM
WBC in (10^3/µL)	5.738	± 0.035	7.456	± 0.066
Lymphocytes (10^3/µL)	1.520	± 0.016	2.75	± 0.033
MID (10^3/µL)	0.433	± 0.075	0.704	± 0.007
Granulocytes (10^3/µL)	3.9	± 0.017	4.433	± 0.092
Lymphocytes (%)	27.066	± 0.082	35.511	± 0.284
MID (%)	5.275	± 0.065	8.445	± 0.133
Granulocytes (%)	67.606	± 0.166	54.584	± 0.302
RBC (10^6/µL)	3.826	± 0.029	4.255	± 0.033
HGB in (g/dL)	10.116	± 0.116	12.277	± 0.148
HCT	44.179	± 0.241	36.291	± 0.146
MCV	118.896	± 0.242	84.045	± 0.227
MCH	40.470	± 0.206	30.334	± 0.140
MCHC	33.728	± 0.153	35.476	± 0.243
RDW-CV	15.87	± 0.148	14.144	± 0.116
RDW-SD	65.623	± 0.137	42.569	± 0.178
PLT (10^3/µL)	150.051	± 0.375	232.85	± 1.134
MPV	13.886	± 0.163	11.478	± 0.118
PCT	2.106	± 0.024	0.279	± 0.003
PDW	16.274	± 0.183	13.463	± 0.093
P-LCC	76.363	± 0.224	91.5	± 0.239
P-LCR	51.041	± 0.164	38.684	± 0.192

WBC: White blood cells, MID: Middle, RBC: Red blood cells, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red cell distribution width-coefficient of variation, RDW-SD: Red cell distribution width - standard deviation, PLT: Platelets, MPV: Mean platelet volume, PCT: Platelet crit, PDW: Platelet distribution width, P-LCC: Platelet large cell coefficient, P-LCR: Platelet larger cell ratio.

Clinically, BMI act as a predisposing risk factor for several bone diseases. Increased BMI in OF group expressing the higher susceptibility towards bone fractures and obesity than NOF counterpart. Weight loss activity may be preventive strategy in osteoporosis management.

Comparison of bone mineral density

According to DEXA report, the BMD was measured to diagnose osteoporosis status and fracture risk between study groups. The mean value of BMD in T-score with SEM was expressed for all OF and NOF volunteers in (**Table 1**). The T-score (≤2.5 SD) were considered for osteoporotic risk. Comparatively, osteoporotic females reported lower BMD (-2.77 ± 0.021) whereas normal in NOF (-0.72 ± 0.023) group and significant differences were observed (P-0.0001). The reduced bone minerals indicate the severity of bone fragile and fracture risk in osteoporotic females.

Hematological assessment and osteoporosis

Hematological data may provide primary input in patients references to bone diseases. In (**Table 2**), the normality of the variables was assessed to link with osteoporosis. All hematological parameters were not found normally distributed among OF and NOF volunteers. To conduct comparative observation between OF and NOF, the average value of WBC, Lymphocytes, MID, Granulocytes, RBC, HGB, MCHC, PLT, and P-LCC were reported lower in females experiencing osteoporosis (5.738 vs 7.456, 1.52 vs 2.75, 0.433 vs 0.704, 3.9 vs 4.433, 3.826 vs 4.255, 10.116 vs 12.277, 33.728 vs 35.476, 150.051 vs 232.85, and 76.363 vs 91.5). In contrast, HCT, MCV, MCH, RDW-CV, RDW-SD, MPV, PCT, PDW, and P-LCR were reported high-

Table 3. Categorization of osteoporotic females and non-osteoporotic females in homocysteine risk categories

Homocysteine risk categories	Osteoporotic females (cases) n=77	Non-osteoporotic females (controls) n=79
Low (<16 µmol/L)	05 (06.49%)	73 (92.40%)
Moderate (16 to 30 µmol/L)	43 (55.84%)	06 (07.60%)
Intermediate (31 to 100 µmol/L)	29 (37.66%)	00 (00.00%)
Severe (>100 µmol/L)	00 (00.00%)	00 (00.00%)

Values in parenthesis represent the value of the cell as a percent of the total n of the column.

Table 4. Measurement of biochemical markers in osteoporotic females and non-osteoporotic females

Biochemical measures	Osteoporotic females (cases) n=77		Non-osteoporotic females (controls) n=79		*p*-value at (<0.05)
	Mean	SEM	Mean	SEM	
Creatinine (mg/dL)	0.670	± 0.012	0.587	± 0.011	0.0001***
Uric Acids (mg/dL)	5.118	± 0.083	2.786	± 0.060	0.0001***
Vitamin B12 (pg/mL)	148.883	± 2.192	294.14	± 6.505	0.0001***
Vitamin D (ng/mL)	24.98	± 0.621	33.7	± 0.652	0.0001***
Homocysteine (µmol/L)	27.322	± 0.816	10.152	± 0.381	0.0001***

*** indicates highly statistically significant at (P<0.05).

er in OF group than their NOF counterpart (44.179 vs 36.291, 118.896 vs 84.045, 40.47 vs 30.334, 15.87 vs 14.144, 65.623 vs 42.569, 13.886 vs 11.478, 2.106 vs 0.279, 16.274 vs 13.463, and 51.041 vs 38.684). Aforementioned variables were found differentially expressed (positively or negatively) along with the low BMD and osteoporotic severity. Adequate proportion of RBC and HGB are so important in maintaining the gaseous (O_2 and CO_2) levels in cells. Hypoxia may disturb cellular physiology, homeostasis and other consequences like bone resorption. Moreover, to protect cellular architecture and biochemistry of bone cells, WBC play central role. Long term up or down the concentration of certain blood components may direct link with bone diseases.

Association of homocysteine with osteoporosis

In clinical aspects, Hcy is a predictive risk marker in bone disorders, cardiovascular disorders, cerebrovascular disorders, neurodegenerative illness, metabolic problems, etc. As expressed in (**Table 3**), all study volunteers (OF and NOF) have been categorized in low, moderate, intermediate, and severe Hcy risk categories. Approximately 55.84% (n=43) of OF expressed moderate, and 37.66% (n=29) expressed intermediate Hcy concentration whereas only 06.49% (n=05) showed low.

Comparatively, in NOF group, nearly 92.40% (n=73) of volunteers were placed in low, and 07.60% (n=06) in moderate Hcy category respectively. Thus intermediate category of NOF group and severe category of both groups were observed completely vacant. The severity of osteoporosis increases in female patients with elevating Hcy concentration.

Association of biochemical markers with osteoporosis

In biochemical measurements, the creatinine, UA, vitamin B12, vitamin D, and Hcy were quantify to establish the association with osteoporotic bone disease (**Table 4**).

Creatinine (P-0.0001) was measured significantly higher in OF group whereas UA (P-0.0001) was reported nearly two times higher in the same group (**Figures 2**, **3**). Increased creatinine and UA can be treated as the markers for several other complications in osteoporotic females.

Further, significant differences were evaluated in existing three variables (vitamin B12, vitamin D, and Hcy) between the study groups. In OF

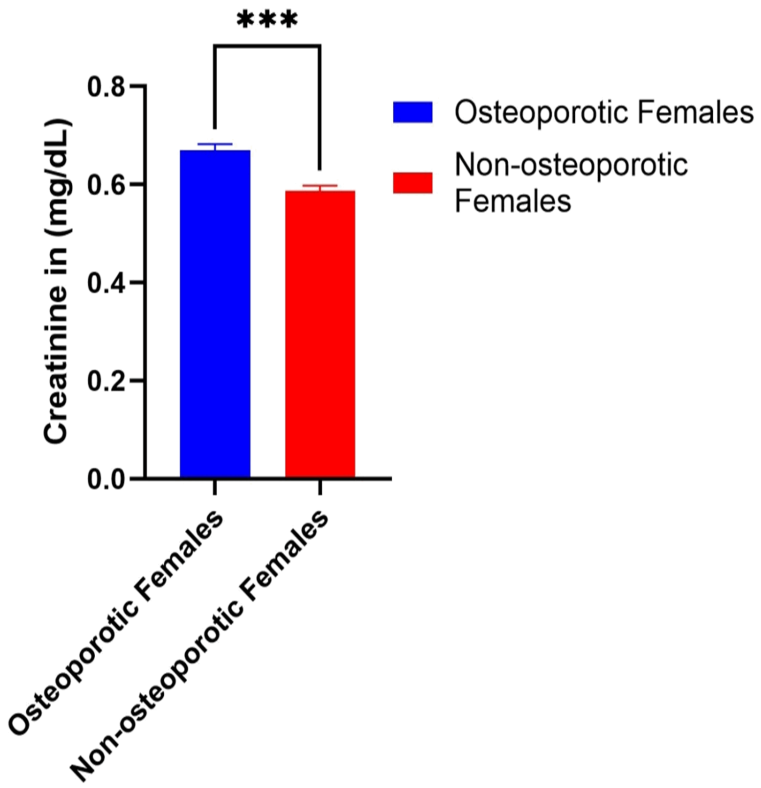

Figure 2. The comparison of Creatinine concentration between OF and NOF groups are shown. A slightly elevated Creatinine (0.670 ± 0.012 vs 0.587 ± 0.011) level was observed in the osteoporotic group. The differences were found statistically significant (P-0.0001) at (P<0.05). *** indicates highly statistically significant at (P<0.05).

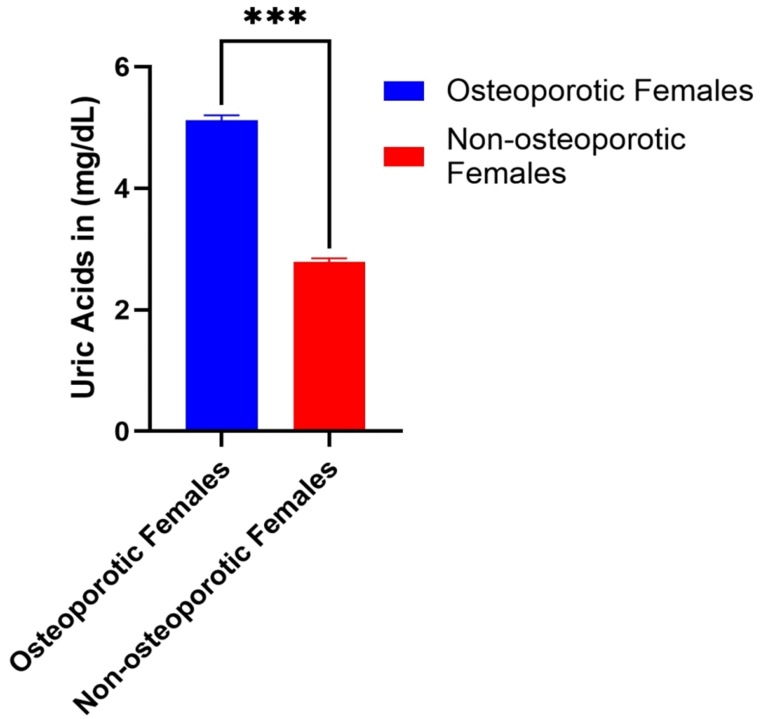

Figure 3. In graphical representation, nearly two times higher UA was reported in OF group (5.118 ± 0.083) than non-osteoporotic group (2.786 ± 0.060). Differences was found statistically significant (P-0.0001) at (P<0.05). Comparatively, high UA may clinically correlate with osteoarthritis, stone formation, and kidney failure in osteoporotic group. *** indicates highly statistically significant at (P<0.05).

group, the concentration of vitamin B12 (P-0.0001) and vitamin D (P-0.0001) were found significantly reduced than NOF counterpart, the same is shown in (**Figures 4**, **5**).

In Hcy quantification report, significantly increased Hcy (P-0.0001) levels were reported in OF group. Contrarily, the NOF expressed a lower value of Hcy (**Figure 6**). In pathological prospective, the quantified parameters including Creatinine, UA, and Hcy were found linked positively with low BMD and osteoporosis. Consistently, reduced BMD, increased severity of osteoporosis were associated with elevated levels of creatinine, UA, and Hcy. Therefore vitamin B12 and vitamin D were reported negatively linked with osteoporosis risk in OF population.

Discussion

The findings of this study highlight the significant role of elevated homocysteine (Hcy) levels in the pathogenesis of osteoporosis in Indian adult females. Our results demonstrate a strong correlation between hyperhomocysteinemia (Hhcy) and reduced bone mineral density (BMD), which aligns with previous studies

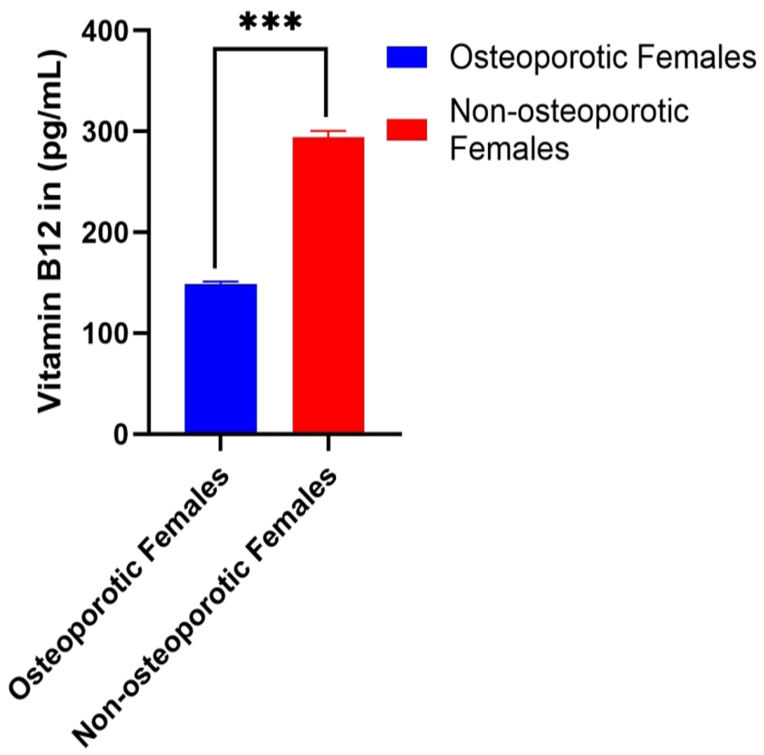

Figure 4. Significantly reduced vitamin B12 concentration was measured in OF (148.883 ± 2.192) volunteers whereas it was reported normal in NOF (294.14 ± 6.505) volunteers. Statistically significant differences were found (P-0.0001) at (P<0.05). In clinical perspective, vitamin B12 plays crucial role in Hcy metabolism and bone health. Low/deficiency of Cobalamin (vitamin B12) defects several metabolic pathways and induces lifelong medical complications in the body. *** indicates highly statistically significant at (P<0.05).

linking elevated Hcy levels to bone demineralization and increased fracture risk. However, our study provides novel insights into the biochemical markers that accompany elevated Hcy, such as creatinine, UA, vitamin B12, and vitamin D levels, and their collective impact on bone health [30, 31].

Elevated Hcy as a predictive marker for osteoporosis

One of the key findings of our study is the strong association between elevated Hcy levels and osteoporosis risk. Females with diagnosed osteoporosis exhibited significantly higher levels of Hcy compared to their healthy counterparts. The role of Hcy in bone metabolism is multifaceted. Hcy interferes with collagen cross-linking in the bone matrix, weakening the extracellular matrix and reducing bone strength. This finding suggests that Hcy may serve as a predictive marker for osteoporosis, potentially allowing for earlier detection and intervention [2, 6, 32-34].

While previous studies have explored the relationship between Hcy and bone health, our study focuses specifically on Indian females, a population that faces unique genetic, nutritional, and environmental challenges. The higher prevalence of Hhcy in this population may be due to deficiencies in key vitamins like B12 and D, both of which are essential for Hcy metabolism. These deficiencies are common in the Indian diet, particularly among women, due to socio-economic and dietary factors. Therefore, the elevated Hcy levels observed in our study could be partially attributed to nutritional inadequacies, further exacerbating the risk of osteoporosis supported the study [35].

Vitamin B12 and vitamin D deficiency

Our study also highlights the critical role of vitamin B12 and vitamin D in regulating Hcy levels and maintaining bone health. Vitamin B12 acts as a cofactor in the remethylation of Hcy to methionine, thus preventing the accumulation of Hcy in the blood. Similarly, vitamin D plays a crucial role in bone remodeling by regulating calcium and phosphate metabolism. The significantly lower levels of both vitamins in osteoporotic females suggest a dual impact: increased Hcy levels due to impaired remethylation and compromised bone strength due to vitamin D deficiency [36, 37].

These findings emphasize the importance of nutritional interventions in the management of osteoporosis. Vitamin B12 and D supplementation could be a cost-effective strategy to reduce Hcy levels and improve bone mineralization, particularly in populations with high rates of deficiency. Given the widespread prevalence of these deficiencies in Indian women, addressing them could significantly reduce the burden of

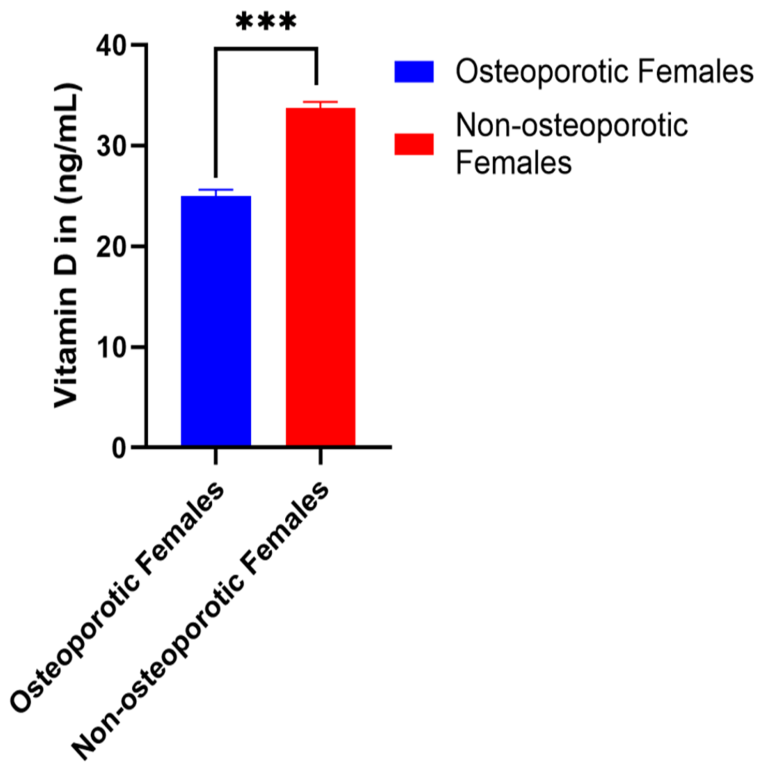

Figure 5. Vitamin D concentration is very important for bone homeostasis. In comparison, lower concentration of vitamin D was measured in OF group (24.98 ± 0.621) while the members of NOF group (33.7 ± 0.652) were expressed normal concentration. This was found statistically significant (P-0.0001) at (P<0.05). Vitamin D concentration may be a predictive risk marker for bone health and osteoporosis. ***Indicates highly statistically significant at (P<0.05).

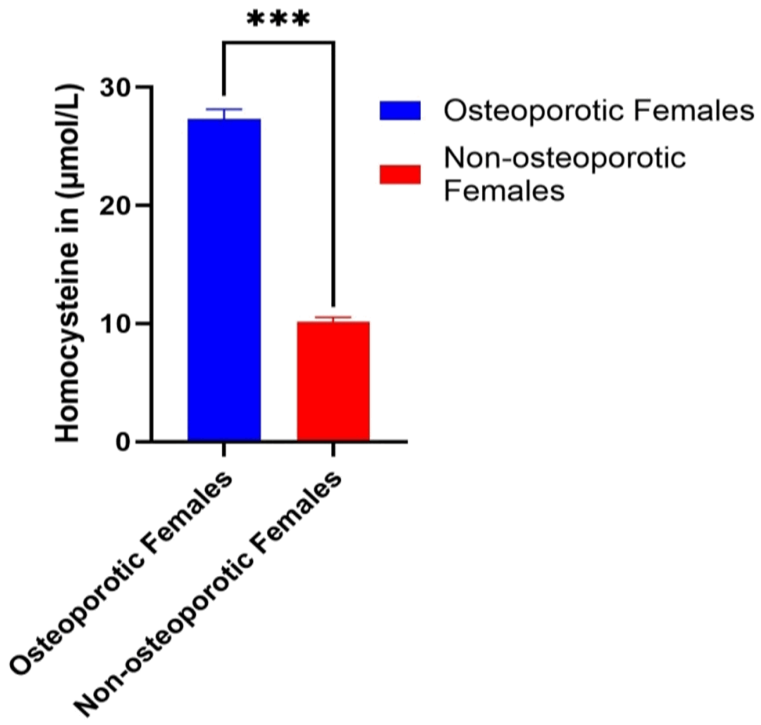

Figure 6. The data of Hcy measurement were expressed: Approximately the mean value of Hcy in OF group (27.322 ± 0.816) was three times higher than its non-osteoporotic counterpart (10.152 ± 0.381). Most OF volunteers were linked to moderate (55.84%) and intermediate (37.66%) Hcy risk categories. A statistically significant difference between the study groups was found (P-0.0001) at (P<0.05). In observation, Hcy levels and osteoporosis risk were found linked directly. *** indicates highly statistically significant at (P<0.05).

osteoporosis in this population.

Creatinine and UA as additional risk factors

In addition to elevated Hcy levels, our study found significantly higher creatinine and UA levels in osteoporotic females. Elevated creatinine may indicate impaired renal function, which has been linked to altered calcium and phosphate homeostasis, further contributing to bone loss. UA, on the other hand, is often associated with oxidative stress and inflammation, both of which play a role in bone resorption. These findings suggest that creatinine and UA, alongside Hcy, could serve as additional biochemical markers for osteoporosis risk [38, 39].

Clinical and therapeutic implications

The findings of this study underscore the importance of a multi-faceted approach to osteoporosis management, particularly in populations at high risk for nutritional deficiencies. Monitoring Hcy levels, along with creatinine, UA, vitamin B12, and vitamin D, could provide a more comprehensive risk assessment

for osteoporosis. Furthermore, targeted nutritional interventions, such as supplementation with vitamin B12 and vitamin D, could potentially mitigate the negative effects of elevated Hcy on bone health.

From a clinical perspective, this study highlights the need for personalized treatment strategies for osteoporosis, particularly in populations with unique dietary and genetic profiles. The integration of Hcy monitoring into routine osteoporosis screening could allow for earlier detection and intervention, potentially reducing the incidence of fractures and improving quality of life for at-risk individuals.

Future directions

While our study provides valuable insights into the relationship between Hcy and osteoporosis, further research is needed to explore the underlying mechanisms by which elevated Hcy contributes to bone loss. Longitudinal studies examining the effects of Hcy-lowering interventions, such as vitamin supplementation, on BMD and fracture risk would be particularly valuable. Additionally, investigating the role of genetic factors, such as polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene, could provide a deeper understanding of individual susceptibility to Hhcy and osteoporosis.

Conclusions

This study demonstrates that elevated homocysteine (Hcy) levels are significantly associated with reduced bone mineral density (BMD) and an increased risk of osteoporosis in Indian adult females. Along with Hcy, deficiencies in vitamin B12 and vitamin D further contribute to bone demineralization, highlighting the critical role of nutritional factors in osteoporosis. Elevated creatinine and UA levels also appear as additional biochemical markers linked to osteoporosis risk.

These findings suggest that Hcy can serve as a valuable predictive marker for osteoporosis, particularly in populations prone to vitamin deficiencies. Implementing nutritional interventions, such as vitamin B12 and D supplementation, may be an effective strategy to reduce Hcy levels and improve bone health. Further research is needed to explore Hcy-lowering interventions and their potential to mitigate osteoporosis progression and reduce fracture risk.

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Informed consent was obtained from all participants individually considered in the study.

Disclosure of conflict of interest

All authors declared no potential conflict of interest concerning the research, authorship, and/or publication of this article.

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