http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.602.611

Research Article
Dietary Pattern and Bone Health in Pre and Post-menopausal Obese Women

[1]Nayera E. Hassan, [2]Salwa M. El Shebini, [2]Nihad H. Ahmed, [1]Sahar A. El-Masry, [1]Safenaz Y. El Sherity, [3]Enas A. Rasheed, [4]Gamila S. El-Saeed and [1]Ayat N. Kamal

[1]Department of Biological Anthropology, National Research Centre, 33 El-Bohooth Street, Dokki, Giza, 12622 Cairo, Egypt
[2]Department of Nutrition and Food Science, National Research Centre, Giza, Egypt
[3]Department of Clinical Pathology, Medical Research Division, National Research Centre, Giza, Egypt
[4]Department of Medical Biochemistry, National Research Centre, Giza, Egypt

Abstract

Background and Objective: Obesity and osteoporosis are worldwide health problems that interact with each other. There are also affected by the menopause and dietary pattern. So, this study aimed to find the relation between osteoporosis, body weight and intake of protein, calcium and vitamin D in obese pre and post-menopausal women. **Materials and Methods:** One hundred and sixteen shared as volunteers in a cross-section study lasted for 2 years. They were divided into 2 groups, pre and post-menopausal women. All women were subjected to, clinical examination, anthropometric measurements and 24 dietary recalls. They were evaluated for bone mass density, biochemical analysis for serum lipids, calcium and vitamin D. **Results:** Osteopenia and osteoporosis were higher among normal-weight and overweight (non-obese) women compared to obese as well weakly associated with their serum lipids. The mean daily protein consumption was high as compared to recommended daily allowances (RDAs), especially among osteoporotic women. The mean daily intake of vitamin D and calcium was low as the lower level was noticed among the osteoporotic premenopausal patients. The means serum concentration of calcium and vitamin D were adequate. **Conclusion:** Data revealed that the prevalence of osteoporosis was lower among obese patients compared to non-obese women. Inadequate daily dietary intake of calcium and vitamin D was reported, however, physiological compensation maintained their optimal normal serum levels.

Key words: Osteoporosis, osteopenia, menopause, obesity, lipid profile, dietary intake, serum lipids, premenopausal patients

Citation: Nayera E. Hassan, Salwa M. El Shebini, Nihad H. Ahmed, Sahar A El-Masry, Safenaz Y. El Sherity, Enas A. Rasheed, Gamila S. El-Saeed and Ayat N. Kamal, 2020. Dietary pattern and bone health in pre and post-menopausal obese women. Pak. J. Biol. Sci., 23: 602-611

Corresponding Author: Sahar A. El-Raufe El-Masry, Department of Biological Anthropology, National Research Centre, 33 El-Bohooth Street, Dokki, Giza, 12622 Cairo, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity and osteoporosis, both are worldwide health problem, the two conditions have been proposed to be the result from deregulations of a common precursor cell, that is, bone marrow mesenchymal stromal cells[1]. The expanding incidence of osteoporotic fractures combined with the absence of information about the disease in the general population means that the disease is consistently expanding, so the increasing burden on health care services are already considerable[2].

The pathogenesis of osteoporosis is an abnormality in typical bone turnover including an imbalance between the procedures of bone resorption and bone formation. Bones become fragile because of a disturbance of its architecture, bringing about an increased danger of fracture. For the most part, bone fragility occurs over a long time and its pathogenesis can happen without notice[3].

Akkawi *et al.*[4] stated that the etiology of osteoporosis are ageing, female sex, low vitamin D and low calcium intake, low body mass index (BMI), abuse immobilization, current smoking and long-term use of certain medications.

It is important to analyze the relationship between dietary aspects and bone health risks. Wang *et al.*[5] reported that vitamin D has an important role in mineralization of bone through vitamin D, calcium and phosphate homeostasis. Hypovitaminosis-D has turned into a pandemic, being seen in all ethnicities and age groups around the world. Ecological components, for example, raised air contamination, diminished ultraviolet B (UVB) irradiation and also lifestyle factors, i.e., decreased open air exercises and poor intake of vitamin D-rich foods, all of these causes were observed with a decreased level of serum vitamin-D[6]. A cross-sectional study was conducted on 404 Egyptian females, to study vitamin-D status in different age groups. The results show a high prevalence of vitamin-D deficiency among healthy Egyptian females especially among elderly female reaching 77.2% in the geriatric group[7].

Impressive consideration has recently focused on the dietary protein's function in the development of the skeleton. Previous investigations reported that high protein consumption is related to lower bone mineral substance, while current studies demonstrate no unfavourable impacts of higher protein consumption[8,9].

Believing that obesity plays a role in the protection of osteoporosis has come into question. Reid reported that body fat and lean mass are correlated with bone mineral density as obesity was conferring protection against bone loss after menopause[10]. However, the most recent epidemiologic and clinical examinations have demonstrated that an abnormal state of fat mass may be a hazard factor for osteoporosis and fractures. The further proof appears to show that criteria of the metabolic syndrome, i.e., hypertension, high level of triglycerides, decreased high-density lipoprotein cholesterol are additionally potential hazard factors for the low bone mineral and osteoporosis[11].

This study aimed to find the relation between osteoporosis and daily dietary intake of calcium, vitamin-D and protein in obese pre and post-menopausal women.

MATERIALS AND METHODS

Sample size: Based on the previous study, 53 subjects in each group were adequate 2 achieve 95% power to detect a difference between the group proportions. The sample size was calculated according to the proportional of obesity in association with osteoporosis regarding the menopausal state. Assuming $\alpha = 0.05$, $B = 0.04$ and power of 95.2%, by Hintze[12].

Study area: One hundred and sixteen women shared as volunteers in a cross-section study which carried out at "Management of visceral obesity and growth disturbances Unit" at the "Medical Research Center of Excellence (MERC)", National Research Centre, Egypt, from January, 2017-October, 2018. They were recruited and randomly chosen, from all employees and workers of all categories, of the "National Research Centre". The Inclusion criteria were that, participants were not suffering from any critical health problems like diabetes mellitus, cardiovascular, thyroid, parathyroid, adrenal, hepatic or renal diseases, also they were not suffering from any diseases that might affect bone health and the inflammatory markers in the past 2 years. Although, they didn't receive hormone replacement therapy, drugs or nutritional supplements that affect bone metabolism or the evaluated biochemical variables.

The Physical activity evaluation (e.g., walking, running, swimming) and external sun exposure (time and period) were recorded. Also, demographic parameters and personal histories, such as the age of menopause, coffee intake, calcium, vitamin D supplement and history of fragility fracture were included.

The final data set of the participants who completed the health examination was divided into 2 groups (according to menopausal state)[13], 54 (46.55%) pre-menopausal and 62 (53.45%) post-menopausal with a mean age of 42.05±8.25 and 51.13±5.82 years and mean BMI of 30.83±8.18 and 34.24±8.80 kg m^{-2}, respectively. Women were considered to

be post-menopausal if they were ≥55 years or didn't have a menstrual period in the last 12 months. All women were subjected to thorough clinical examination. Each group divided into 3 groups: normal, osteopenia (non-osteoporotic) and osteoporosis according to their bone health status. Bony mass density (BMD) (g cm^{-2}) was measured in the total hip (femoral neck) and lumbar spine (L2–L4) by dual-energy X-ray absorptiometry (DEXA) with (Norland Xr-46, with host software version: 3.9.6/2.3.1., USA). The instruments were calibrated daily according to the manufacturer's instructions. Osteoporosis is established by measurement of BMD of the hip and spine using the T-score which was calculated using the following formula:

$$\text{T-score} = \frac{\text{Measured bone density} - \text{Maximum bone density}}{\text{Maximum standard deviation}}$$

if T-score ≥ -1.0 was grouped as normal, T-score < -1.0 to >-2.5 was put in the osteopenia and T-score ≤-2.5 were categorized as having osteoporosis following the diagnostic criteria established by the World Health Organization (WHO)[14].

The protocol of the study was approved by the National Research Centre Ethics Committee number 16/127 through a project titled "Bone mass among overweight and obese women: Mechanism and Intervention". Although, informed written consent was obtained from each participant to be included in the study. Data collected in "Management of visceral obesity and growth disturbance unit" in the Medical Research Centre of Excellence (MRCE)-National Research Centre.

Anthropometric parameters: Full clinical examination was carried out which included blood pressure, chest, heart, abdominal and central nervous system examination. Then Relevant anthropometric measurements were recorded including height and weight using standard methods following the recommendations of the International Biological Program[15]. Three consecutive measurements were taken and when the differences between the readings were acceptable the mean was recorded. Body weight was measured by using Seca scale and approximated to the nearest 0.01 kg with minimal clothes for which no correction was made, height without shoes using Holtain portable Stadiometer and approximated to the nearest 0.1 cm. Then, BMI was calculated weight (kg)/height (cm^2). The sample was classified to 3 groups: normal (BMI =18-<25), overweight (BMI ≥25-<30) both consider as (non-obese) and obese (BMI ≥30). Among the pre-menopausal women 22.23, 16.67 and 61.11% were normal-weight, overweight and obese respectively, while the frequency in the post-menopausal women were 19.36, 14.52 and 66.13%, respectively.

Blood sampling and biochemical analysis: Fasting blood samples (after 12 h fasting) were drawn from the patients. Biochemical parameters were performed on fasting sera that were stored at -70°C until used. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured using commercially available kits provided by Stanbio Laboratory Inc. (1261 North 18 Main Street Boerne Texas 78006 USA). Low-density lipoprotein-cholesterol (LDL-C) was calculated according to an equation developed by Friedewald *et al.*[16] as follows:

$$\text{LDL} - \text{C} = \frac{\text{Total cholesterol} - \text{Triglycerides}}{5 + \text{HDL} - \text{C}}$$

Serum calcium was determined using the automated clinical chemistry analyzer Olympus AU 400 analyzer. Serum 25 hydroxy vitamin-D (25 (OH) D) was assessed by ELISA kit, for Vitamin-D Catalogue number SL1831 HU. Sun long Biotech Co. Ltd., all were done in the laboratory of NRC in Egypt.

Dietary recalls: Information from each woman about her usual pattern of food intake was obtained. Data was collected through a dietary interview consisting of a 24 h recall that repeated for 3 days. Analysis of food items was done using World Food Dietary Assessment System, (WFDAS), 1995, USA, University of California[17].

Statistical analysis: All values were expressed as mean value±SD, two-tailed student t-test was used to compare between different phases in the same group. Correlation between the different parameters was tested by the Pearson test. The $p<0.05$ were considered statistically significant also chi-square test, scatter plot graphs and finally odd ratio for the risk factor. SPSS window software version 17.0 (SPSS Inc. Chicago, IL, USA, 2008)[18] was used.

RESULTS

Comparisons between pre and post menopausal women: Table 1 shows the mean±SD of age, height, weight and BMI of pre and post-menopausal women. The post-menopausal women were older, while the BMI was higher among pre-menopausal.

Table 1: Mean±SD of age, weight, height and BMI of pre and post-menopausal women

Parameters N (116)	Pre-menopausal No. (54) Mean±SD		Post-menopausal No. (62) Mean±SD	
Age (Year)	42.05±8.25		51.13±5.82	
Weight (kg)	76.96±2.27		80.15±1.90	
Height (m)	1.58±5.59		1.53±6.73	
BMI	30.83±8.18		34.24±8.80	
Body mass index (kg ht^{-2})	Non obese No. (%)	Obese No. (%)	Non obese No. (%)	Obese No. (%)
	21 (39)	33 (61)	21 (34)	41 (66)

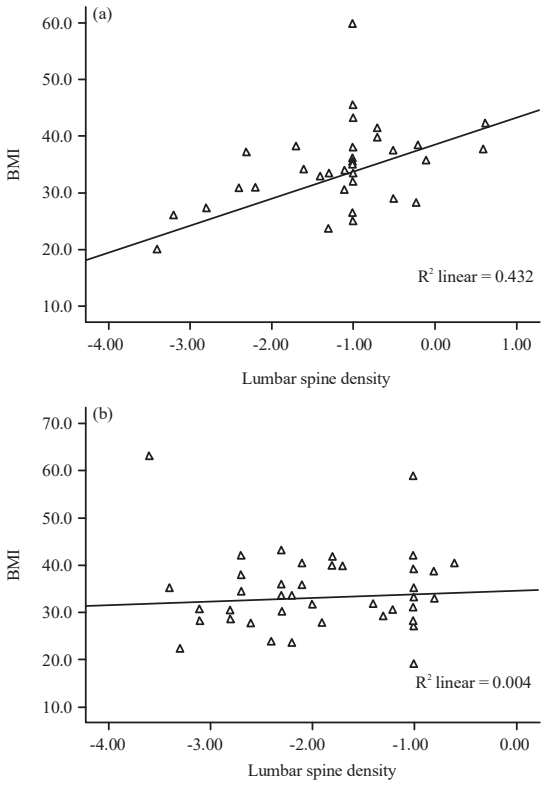

Fig. 1(a-b): Scatter plot graph for the association between lumbar spine density and BMI according to menopausal state (a) pre-menopause and (b) post-menopause

(a) Pre-menopause women had a highly significant association between increasing BMI and normal lumbar bone density (r = 0.422), (b) Post-menopausal women had a weak significant association (r = 0.006) for the spine density

Table 2 shows the mean and SD of the daily nutrient intake and RDAs percentage for pre and post-menopausal women according to their BMI. Data revealed that, the daily intake of calories, protein, fat and cholesterol among the obese pre and post-menopausal women was high when compared to the RDAs in both groups, with significant difference between obese and normal-weight women at $p \leq 0.05$. The daily intake of carbohydrate was significantly high among obese post-menopausal women. The daily intake of vitamin A and D of the entire studied sample was low compared to the RDAs. Significant differences were detected between obese and normal-weight women at $p \leq 0.05$. The daily intake of the sodium and potassium was adequate, while that of calcium, iron and zinc was low compared to the RDAs.

Table 3 shows the mean±SD of daily intake of protein, carbohydrate, fat, vitamin-D and calcium and their RDAs percent for the pre and post-menopausal women according to bone mass density. The daily protein consumption of the pre and post-menopausal women in the three groups was high compared to the RDAs especially among the osteoporotic patients in both groups, significant differences at $p \leq 0.05$-0.01 were detected between the normal and the other 2 groups. Carbohydrate and fat daily intake of the osteoporotic pre and post-menopausal women was significantly high compared to other groups. The daily intake of vitamin-D and calcium was low compared to the RDAs, lowest levels were found among both the osteoporotic pre and post-menopausal women, with significant difference ($p \leq 0.05$) only in the last group.

Table 4 shows the association between BMI and lumbar spine and femur densities among pre- and post-menopausal women. Data revealed that all obese pre-menopausal women were non osteoporotic at both sites compared to 47 and 53% and 38 and 62% of the non-obese women respectively, with significant difference($p \leq 0.000$). Also significant differences were present with the post-menopausal women with p-value (0.055 and 0.058) for the spine and femur density respectively.

Relation between obesity and BMD at lumbar spine and femur head: Table 5 shows the values of odds ratio between obesity and lumber spine and femur densities in pre- and post-menopausal women. Data revealed a statistical significance values by the 95% confidence interval (CI) with high precision at both pre and post-menopause women, more prominent at pre-menopausal.

Figure 1 and 2 show scatter plot graphs, for the association between BMI with lumbar spine and femur densities according to menopausal state (pre-menopause (a) and post-menopausal (b)), there were highly significant

Table 2: Daily nutrients intake (mean±SD) and the RDA (%) for the pre and post-menopausal women according to their BMI

	Parameters												
	Pre-menopausal (54)						Post-menopausal (62)						
	Normal		Overweight		Obese		Normal		Overweight		Obese		
Nutrient intake	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	RDAs
Energy (kcal)	1062.58±30.83	53.13	1637.92±23.13	81.90	2458.23±34.52^{b**,c*}	122.76	1202.15±38.12	60.11	2269.42±34.52	113.47	2912.36±32.72^{b**,c*}	145.62	2000
Protein (g)	46.35±13.56	92.70	59.86±12.66^{a*}	119.72	76.46±14.52^{b*}	152.64	47.91±12.87	95.82	88.79±20.42^{a*}	177.58	89.68±21.43^{b*}	179.36	50
Carbohydrate (g)	123.11±23.41	44.77	210.64±31.53	76.59	238.864±30.72^{b*}	83.64	149.94±24.14	54.52	252.73±31.60^{a*}	91.90	368.77±30.51^{b**}	134.09	275
Dietary fiber (g)	26.12±4.22	104.48	23.40±12.03	93.60	21.01±10.12	80.56^{b*}	24.19±4.33	96.76	18.11±5.20	72.44^{a*}	14.80±4.20	59.20^{b*}	25
Fat (g)	42.75±11.80	55.52	61.77±12.24	80.22	112.26±14.26^{b**,c*}	145.79	45.64±12.71	59.27	100.37±21.16^{a*}	130.35	119.51±31.16^{b**,c*}	155.21	77
SFA (g)	16.21±4.11		31.92±6.19		54.89±5.14^{b**c*}		20.43±4.01		49.97±11.18 ^{a*}		58.77±11.14^{b**,c*}		
MUFA (g)	12.90±3.26^{b*}		9.75±6.13		8.19±3. 51		12.11±2.41		19.24±12.14		7.02±3.10^{c*}		
PUFA (g)	12.05±3.61		8.10±5.11		6.52±5.20^{b*}		11.51±2.16		14.17±10.01		5.20±2.04^{b*c*}		
Cholesterol (mg)	214.96±30.41	71.65	415.16±34.17^{a*}	138.39	423.12±33.14^{b*}	141.04	232.40±25.44	77.47	438.76±26.21^{a*}	146.25	486.75±24.43^{b**}	162.25	300
Vitamin A (µg)	651.31±33.51	81.42	641.73±42.16	80.22	616.55±38.19	77.07^{b*c*}	632.72±30.18	79.09	624.23±31.71	78.03	578.27±26.82	72.28	800
Vitamin D (µg)	3.23±1.12	64.60	2.17±1.15	43.40^{a*}	2.01±1.05	40.20^{b*}	3.06±1.02	60.20	2. 12±0.81	42.40	1.95±0.76^{b*}	39.00	5
Sodium (mg)	1528.13±21.14	101.88	1589.41±20.25	105.96	1639.32±22.34	109.29^{b*}	1539.30±33.41	102.62	1679.02±32.23^{a*}	111.93	1710.82±38.11^{b*}	114.01	1500
Potassium (mg)	2126.26±31.55	106.31	2270.61±30.19	113.53	2249.82±32.60	112.49	2084.41±24.16	104.22	2240.36±27.40	112.02	2218.21±34.70	110.91	2000
Calcium (mg)	756.32±26.15	75.63	620.16±23.52	62.02^{a*}	641.45±23.19	64.15^{b*}	614.74±31.17	60.47	590.34±35.10	59.03	560.23±31.40	56.02	1000
Iron (mg)	7.36±2.18	49.07	7.33±1.20	48.87	6.16±1.32	41.07^{b*}	7.21±2.34	48.07	6.31±2.19	42.07	6.11±2.20	40.73	15
Zinc (mg)	6.53±1.02	54.42	6.85±1.10	57.08	6.49±1.11	54.08	6.33±2.40	52.75	6.19±2.23	51.58	5.54±2.12	46.17	12
BMI No. (%)	12 (22.23)		9 (16.67)		33 (61.67)		12 (19.36)		9 (14.52)		41 (66.13)		

a: Normal vs. overweight, b: Normal vs. obese, c: Overweight vs. Obese, *Significant at p≤0.05, **High significant at p≤0.01

Table 3: Daily intake of protein, vitamin D, calcium (mean±SD) and their RDA (%) for the pre and post-menopausal women according to bone mass (femur site)

	Parameters												
	Pre-menopausal women No. (54)						Post-menopausal women No. (62)						
	Normal bone mass		Osteopenia		Osteoporosis		Normal bone mass		Osteopenia		Osteoporosis		
Nutrient intake	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	Mean±SD	RDA (%)	RDAs
Protein (g)	40.45±6.32	80.90	54.12±6.26	108.24^{a*}	80.47± 6.22	160.94^{b**,c*}	65.96±8.10	131.92	73.14±11.21	146.28^{a*}	81.03±11.27	162.06^{b**,c*}	50
Carbohydrate (g)	123.021±11.34	44.73	217.46±11.40	79.08^{a*}	225.66±12.52	82.06^{b**,c*}	144.43±12.18	51.09	251.34±13.61	91.39^{a*}	295.13±16.32	107.32^{b**,c*}	275
Fat (g)	47.11±6.30	61.19	67.11±6.44	87.16^{a*}	131.21±.14.37	170.40^{b**c*}	83.97±11.18	109.05	106.80±12.41	138.70^{a*}	129.33±13.27	167.96^{b**,c*}	77
Cholesterol (mg)	218.34±11.36	72.78	410.43±14.57	136.81^{a*}	426.39±12.60	142.13^{b**}	246.63±14.19	82.21	443.71±12.36	147.90^{a*}	469.46±13.31	156.49^{b**,c*}	300
Vitamin D (µg)	3.22±2.31	64.40	2.56±1.23	51.20^{a*}	2.41±1.18	48.20	3.18±2.03	63.60	2.39±1.05	47.80	2.31±1.10	47.20	5
Calcium (mg)	697.19±30.32	69.72	656.27±27.31	65.63^{a*}	595.89±25.41	59.59	690.57±23.16	69.06^{b*}	638.72±22.10	63.87	581.76±23.11	58.18	1000
BM No. (%)	23 (42.59)		18 (33.33)		13 (24.07)		15 (24.19)		29 (46.77)		18 (29.03)		

a: Normal vs. osteopenia, b: Normal vs. osteoporosis , c: Osteopenia vs. osteoporosis, *Significant at p≤0.05, **High significant at p≤0.01

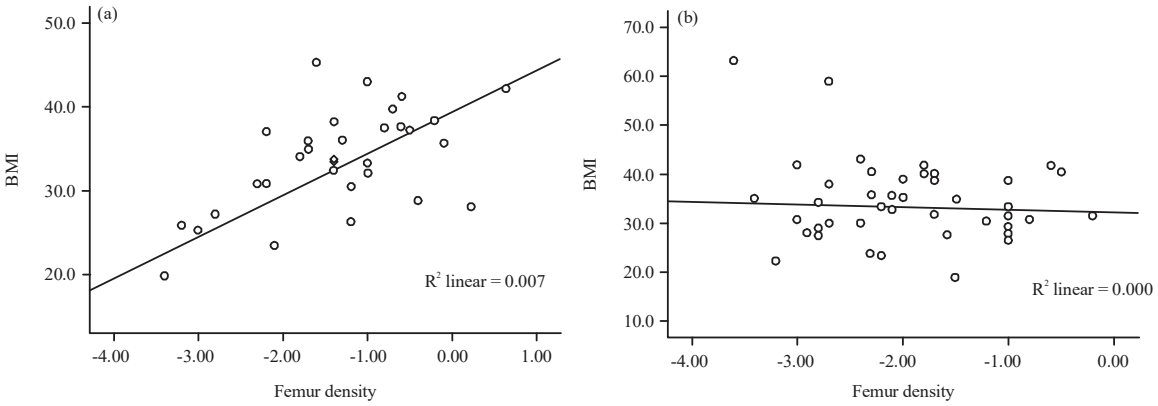

Fig. 2(a-b): Scatter plot graph for the association between femur density and BMI according to menopausal state (a) Pre-menopause and (b) Post-menopause

(a) Pre-menopause women had a highly significant association between increasing BMI and normal femur bone density (r = 0.607) and (b) Post-menopausal women had a weak significant association (r = 0.003) for the femur density

Table 4: Association between BMI and lumbar spine and femur densities in Pre- and post-menopausal women

		Pre-menopausal (No 54)			Post-menopausal (62)		
Sites	Bone status	Non obese No. 21	Obese No. 33	p-value	Non obese No. 21	Obese No. 41	p -value
Spine	Non osteoporosis	10 (47%)	33 (100%)	0.000	13 (62%)	34 (83%)	0.050
	Osteoporosis	11 (53%)	0		8 (38%)	7 (17%)	
Femur	Non osteoporosis	8 (38%)	33 (100%)	0.000	13 (62%)	31 (76%)	0.030
	Osteoporosis	13 (62%)	0		8 (38%)	10 (24%)	

Table 5: Odds ratio to predict effect of obesity as a risk factor on lumbar spine and femur densities in pre- and post-menopausal women

	Pre-menopausal		Post-menopausal	
Sites	Values	95% confidence interval	Values	95% confidence interval
Spine	0.476	0.304-0.746	0.746	0.519-1.073
Femur	0.381	0.221- 0.657	0.819	0.561-1.195

Table 6: Important biochemical parameters for the pre and post-menopausal women according to bone mass (mean±SD)

	Biochemical parameters (Mean±SD)					
	Pre-menopausal women N (54)			Post-menopausal women No. (62)		
Nutrient intake	Normal bone mass	Osteopenia	Osteoporosis	Normal bone mass	Osteopenia	Osteoporosis
Cholesterol (mg L^{-1})	187.85±3.43	189.40±4.25	191.83±3.71	201.47±6.34	221.31±4.44	227.20±6.23^{b*,c*}
TG (mg L^{-1})	90.57±5.14	92.90±4.15	97.42±3.12^{b*}	108.62±6.09	132.78±5.40^{a**}	134.81±4.19^{b**},
HDL (mg L^{-1})	43.50±1.13	42.80±1.44	53.19±1.42^{b*,c*}	41.83±4.17	40.07±5.11	39.64±4.31
LDL-C (mg dL^{-1})	116.72±5.18	119.81±4.61^{a*}	126.84±5.30^{b*}	124.94±5.24	134.23±6.38^{a*}	147.40±5.19^{b*}
Calcium (mg dL^{-1})	9.59±0.84	9.10±0.56	9.07±0.74	9.21±0.31	9.14±0.18	9.02±0.11
Vitamin D (µg dL^{-1})	38.21±6.08	35.20±5.01	24.38±6.40^{b*c*}	39.71±4.64	32.16±3.22^{a*}	23.39±2.15^{b*,c*}
BM	23 (42.59)	18 (33.33)	13 (24.07)	15 (24.19)	29 (46.77)	18 (29.03)

*Significant at $p \leq 0.05$, **High significant $p<0.001$, a: Normal vs. osteopenia, b: Normal vs. osteoporosis, c: Osteopenia vs. osteoporosis

association between increased BMI and normal lumbar and femur bone densities at pre-menopause r = 0.422 and 0.607) while weak significant association for the spine and femur densities at post-menopause (r = 0.006 and 0.003).

Biochemical parameters for the pre and post-menopausal women according to BMD groups: Table 6 shows the mean±SD of important biochemical parameters for the pre and post-menopausal women according to Bone Mass. The means serum concentration reported for the total cholesterol (TC) and the LDL-C were high among the 3 groups of the post-menopausal patients, significant value at $p \leq 0.05$ was detected at the osteoporotic post-menopausal patients. The mean values of the serum triglyceride (TG) were within the normal value, higher significant value was found among the osteoporotic post-menopausal women. The mean serum

Table 7: Correlation coefficient of femur and lumber score with protein intake and serum concentration of calcium, vitamin D, LDL-C and total cholesterol

	p-value	
Parameters	Femur site	Spine site
Protein intake (g)	0.133	0.372
Serum calcium (mg dL^{-1})	-0.129	0.046*
Serum vitamin D (ng dL^{-1})	-0.676	-0.467
Serum LDL-C (mg dL^{-1})	-0.176	0.552
Serum T. cholesterol (mg dL^{-1})	-0.169	0.181

*Significant $p \leq 0.05$

concentrations of the HDL-C were low compared to the standard level, normal range was found among osteoporotic pre-menopausal patients. The means values of serum calcium concentration were within normal ranges, the values were ranged from 9.02 ± 0.74 to 9.59 ± 0.84 mg dL^{-1}. Mean values of serum vitamin D showed significant difference between osteoporotic and both normal and osteopenic women in both groups.

Correlations between BMD at lumbar spine and femur head: Table 7 shows the correlation coefficient of the femur and lumbar spine densities with protein intake and serum concentration of calcium, vitamin-D, total cholesterol and LDL-C. Serum calcium concentration shows a significant positive correlation with the L spine at $p \leq 0.05$ while vitamin-D levels, daily protein intake, total cholesterol and LDL-C show a different weak association.

DISCUSSION

It is known that osteoporosis is a multifactorial disease. In Egypt, 28.4% of post-menopausal women have osteoporosis, while 53.9% have osteopenia[19]. In this study, the effect of obesity through the determination of BMI in addition to the effect of the dietary intake of protein, calcium and vitamin-D on the occurrence of osteoporosis among the pre and post-menopausal women was investigated. The data of this study revealed that osteoporosis prevalence among the studied sample was 20.4 and 24.2% in the lumbar spine, while at the femur site it was 24.1 and 29.0% for pre- and post-menopausal women respectively. Previous research was done by Cui *et al.*[20] reported that osteoporosis prevalence in lumbar spine and femur site were 40.1 and 12.4%, respectively in Koreans women aged 50-79 years.

Conflicting results were obtained about the relation between body weight and osteoporosis. It has been stated that obese women had a lower prevalence of osteopenia compared with normal-weight subjects and also with a lower prevalence of osteoporosis as compared to normal and overweight women[21]. Data of this study are in agreement with this study where the prevalence of osteoporosis was lower among the obese pre and post- menopausal patients compared to the non-obese women. Moreover the odd revealed low associated of obesity with osteoporosis which was more evident at pre-menopausal women. Besides association was detected between the BMI and normal lumbar spine and femur density at the menopausal state (pre-menopause and post-menopausal).

Bonjour[22], reported that sufficient dietary intake assumes to have an important role in the improvement and keep up of bone structures. In this context calcium with a sufficient supply of vitamin-D, dietary proteins all play a critical role for bone wellbeing and along these lines, their deficiencies work in the anticipation of osteoporosis[22].

According to the results reported in the current study both the dietary intake of calcium and vitamin-D was low when compared to the RDAs, however, their serum concentrations were at the normal levels. In this context, Zhang *et al.*[23], said that maintaining the level of circulating ionized calcium within a narrow physiological range is critical for the body to function normally. It is known that extracellular calcium homeostasis is for the most part controlled by 3 physiological modes, including intestinal calcium absorption, renal calcium reabsorption and bone resorption or in other words by calcium-sensing receptor through the regulation of parathyroid hormone (PTH), calcitonin and 1,25-dihydroxy vitamin-D3 secretion[24]. Also studies of bone histomorphometry by Recker *et al.*[25] showed that bone remodelling is quickened in the pre and post-menopausal periods. The range of 5-10 years around menopause is described by a reduction in estrogen secretion and an expansion in resorption of calcium from the bone[26], bringing about a marked diminish in bone density. As for the normal level of serum vitamin-D that reported among our patients despite the low dietary intake, this might attribute to the sun exposure, where it is known that Egypt is a country that enjoys the sunlight throughout the year. Brouwer-Brolsma *et al.*[27] reported that of the most important reason affecting serum concentration of vitamin-D is the sun exposure which still appeared to be an important determinant of serum 25 (OH)D in older individuals, closely followed by genes and vitamin D intake. However intragroup variation was detected among patients in this study as 28.8% had insufficient serum vitamin-D concentration as their levels were ranged from 16-19 ng mL^{-1}. Addition, a negative association was found between serum vitamin-D and osteoporotic scores[27].

Considerable attention has recently focused on dietary protein's role in the mature skeleton. It has been hypothesized that high protein intakes are associated with lower bone mineral content (BMC) as acidic amino acids may promote bone resorption[28]. However, several surveys and meta-analyses discussed the advantages and dangers of dietary protein consumption for bone health in adults, the results revealed that dietary protein levels even over the current RDA might help lessen bone loss given that calcium is satisfactory[29]. The results of this study showed that the protein intake of the osteopenic and the osteoporotic pre and post-menopausal patients was high when compared to the RDAs, in the same time the post-menopausal women who had normal bone density were also consuming high protein, yet the levels of calcium and vitamin-D intake were higher compared to the other groups, which is likely to support the statement of the previous study. Data of this study reported no significant association between both daily protein intake and osteoporosis.

Obese patients usually suffer from lipid abnormality as elevated triglyceride, VLDL, Apo B and non-HDL cholesterol levels, in addition to low HDL cholesterol and Apo A-I levels[30]. In a few investigations, lipid disorders have been associated with low bone mineral density[31]. This association might be straight forwardly related to the cholesterol biosynthetic pathway, which affects cholesterol levels and also related to the activity of the osteoclasts[32]. The standard level of cholesterol is important for the osteoblastic differentiation of marrow stromal cells[33]. Low-density lipoprotein (LDL) receptor-related protein 5 (LRP5) deficient mice are appeared to have both hypercholesterolemia and decrease of bone mass[34]. Change of LRP6 in people was appeared to cause early-beginning of the cardiovascular disease in addition to serious osteoporosis due to high serum LDL-C levels[35]. However, Ghadiri-Anari *et al.*[36] found an unadjusted negative relationship between serum total cholesterol levels with femoral BMD. By using a linear regression adjusted for weight and BMI the authors found no association between serum lipids level and BMD. Additionally, different lipid levels did not show any significant difference between both groups which in agreement with data of this study[36].

A lack of research into osteoporosis is aggravating the situation. National osteoporosis guidelines strategy for diagnosis and management for osteoporosis risk factors reduction should be recommended. An important part of this strategy is to conduct community educational interventions to improve awareness about the seriousness and causes of osteoporosis between the members of society.

CONCLUSION

The results of this study revealed that the prevalence of osteoporosis was lower among obese pre and post-menopausal women compared to the non-obese women and a different weak association with their serum lipid profile. Data showed inadequate daily dietary intake of calcium, however, physiological compensation maintained its optimal normal serum levels at the expense of bone content of calcium. In the pre and post-menopausal women adopting a balanced diet, rich in nutrients, minerals and vitamins can contribute significantly to bone health.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of calcium, vitamin D and sun exposure combination that can be beneficial to minimize the incidence of osteoporosis. Also, obesity take a role as the prevalence of osteoporosis was lower among the Egyptian obese pre and post- menopausal women compared to the non-obese ones. This study will help the researchers to uncover the critical area of postmenopausal bone loss that many researchers were not able to explore. Thus, a new theory on these factors combination and possibly other combinations, may be arrived at.

ACKNOWLEDGMENT

We would like to acknowledge our institute "National Research Centre', Egypt", without its fund this study could not be done. Authors are also grateful to everybody participated in this study, the employers of our institute who were the participants of this study, the technicians who helped in the laboratory analysis and the doctors who participated in collection of the data'. Without their help, this study couldn't have been completed.

REFERENCES

1. Horowitz, M.C. and J.A. Lorenzo, 2004. The origins of osteoclasts. Curr. Opin. Rheumatol., 16: 464-468.
2. Bilal, M., A. Haseeb, A.Z. Merchant, A. Rehman and M.H. Arshad *et al.*, 2017. Knowledge, beliefs and practices regarding osteoporosis among female medical school entrants in Pakistan. Asia Pac. Fam. Med., Vol. 16. 10.1186/s12930-017-0036-4.
3. Manolagas, S.C., 2000. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrinol. Rev., 21: 115-137.

4. Akkawi, I. and H. Zmerly, 2018. Osteoporosis: Current concepts. Joints, 6: 122-127.
5. Hwang, S., H.S. Choi, K.M. Kim, Y. Rhee and S.K. Lim, 2015. Associations between serum 25-hydroxyvitamin D and bone mineral density and proximal femur geometry in Koreans: The Korean National Health and Nutrition Examination Survey (KNHANES) 2008-2009. Osteoporosis Int., 26: 163-171.
6. Caccamo, D., S. Ricca, M. Currò and R. Ientile, 2018. Health risks of hypovitaminosis D: A review of new molecular insights. Int. J. Mol. Sci., Vol. 19, No. 3. 10.3390/ijms19030892.
7. Botros, R.M., I.M. Sabry, R.S. Abdelbaky, Y.M. Eid, M.S. Nasr and L.M. Hendawy, 2015. Vitamin D deficiency among healthy Egyptian females. Endocrinol. Nutri., 62: 314-321.
8. Shams-White, M.M., M. Chung, M. Du, Z. Fu abd K.L. Insogna *et al*., 2017. Dietary protein and bone health: A systematic review and meta-analysis from the National Osteoporosis Foundation. Am. J. Clin. Nutr., 105: 1528-1543.
9. Isanejad, M., J. Sirola, J. Mursu, H. Kröger, M. Tuppurainen and A.T. Erkkilä, 2017. Association of protein intake with bone mineral density and bone mineral content among elderly women: The OSTPRE fracture prevention study. J. Nutr. Health Aging, 21: 622-630.
10. Reid, I.R., 2002. Relationships among body mass, its components and bone. Bone, 31: 547-555.
11. Migliaccio, S., E.A. Greco, R. Fornari, L.M. Donini and A. Lenzi, 2011. Is obesity in women protective against osteoporosis? Diabetes Metab. Syndr. Obes., 4: 273-282.
12. Hintze, J., 2011. PASS 11. NCSS, LLC. Kaysville, Utah, USA.
13. Doventas, A., I.M. Bolayirli, S. Incir, H. Yavuzer and S. Civelek *et al*., 2015. Interrelationships between obesity and bone markers in post-menopausal women with either obesity or osteoporosis. Eur. Geriatr. Med., 6: 15-20.
14. WHO., 2003. Prevention and management of osteoporosis: Report of a WHO scientific group. WHO Technical Report Series No. 921, World Health Organization, Geneva, pp: 1-164.
15. Hiernaux, J. and J.M. Tanner, 1969. Growth and Physical Studies. In: Human Biology: A Guide to Field Methods, Weiner, J.S. and S.A. Lourie (Eds.). International Biological Programme by Blackwell Scientific, London, UK.
16. Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.
17. Anonymous, 1995. World Food Dietary Assessment System, (WFDAS). University of California, USA.
18. SPSS., 2008. SPSS Statistics for Windows. Version 17.0, SPSS Inc., Chicago, IL., USA.
19. El-Tawab, S.S., E.K.A. Saba, H.M.T. Elweshahi and M.H. Ashry, 2016. Knowledge of osteoporosis among women in Alexandria (Egypt): A community based survey. Egypt. Rheumatol., 38: 225-231.
20. Cui, L.H., J.S. Choi, M.H. Shin, S.S. Kweon and KS. Park *et al*., 2008. Prevalence of osteoporosis and reference data for lumbar spine and hip bone mineral density in a Korean population. J. Bone Miner. Metab., 26: 609-617.
21. Mazocco, L. and P. Chagas, 2017. Association between body mass index and osteoporosis in women from northwestern Rio Grande do Sul. Rev. Bras. Reumatol. (English Edn.), 57: 299-305.
22. Bonjour, J.P., 2011. Protein intake and bone health. Int. J. Vitam. Nutr. Res., 81: 134-142.
23. Zhang, C., C.L. Miller, E.M. Brown and J.J. Yang, 2015. The calcium sensing receptor: From calcium sensing to signaling. Sci. Chin. Life Sci., 58: 14-27.
24. Boros, S., R.J. Bindels and J.G. Hoenderop, 2009. Active Ca^{2+} reabsorption in the connecting tubule. Pflügers Archiv-Eur. J. Physiol., 458: 99-109.
25. Recker, R., J. Lappe, K.M. Davies and R. Heaney, 2004. Bone remodeling increases substantially in the years after menopause and remains increased in older osteoporosis patients. J. Bone Miner. Res., 19: 1628-1633.
26. Finkelstein, J.S., S.E. Brockwell, V. Mehta, G.A. Greendale and M.R. Sowers *et al*., 2008. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. J. Clin. Endocrinol. Metab., 93: 861-868.
27. Brouwer-Brolsma, E.M., A.M. Vaes, N.L. van der Zwaluw, J.P. van Wijngaarden and K.M. Swart *et al*., 2016. Relative importance of summer sun exposure, vitamin D intake and genes to vitamin D status in Dutch older adults: The B-PROOF study. J. Steroid Biochem. Mol. Biol., 164: 168-176.
28. Beasley, J.M., L.E. Ichikawa, B.A. Ange, L. Spangler, A.Z. LaCroix, S.M. Ott and D. Scholes, 2010. Is protein intake associated with bone mineral density in young women? Am. J. Clin. Nut., 91: 1311-1316.
29. Rizzoli, R., E. Biver, J.P. Bonjour, V. Coxam and D. Goltzman *et al*., 2018. Benefits and safety of dietary protein for bone health-an expert consensus paper endorsed by the European Society for Clinical and Economical Aspects of Osteopororosis, Osteoarthritis and Musculoskeletal Diseases and by the International Osteoporosis Foundation. Osteoporosis Int., 29: 1933-1948.
30. Bays, H.E., P.P. Toth, P.M. Kris-Etherton, N. Abate and L.J. Aronne *et al*., 2013. Obesity, adiposity and dyslipidemia: A consensus statement from the national lipid association. J. Clin. Lipidol., 7: 304-383.
31. Orozco, P., 2004. Atherogenic lipid profile and elevated lipoprotein (a) are associated with lower bone mineral density in early postmenopausal overweight women. Eur. J. Epidemiol., 19: 1105-1112.
32. Bauer, D.C., 2003. HMG CoA reductase inhibitors and the skeleton: A comprehensive review. Osteoporos. Int., 14: 273-282.

33. Parhami, F., N. Mody, N. Gharavi, A.J. Ballard, Y. Tintut and L.L. Demer, 2002. Role of the cholesterol biosynthetic pathway in osteoblastic differentiation of marrow stromal cells. J. Bone Miner. Res., 17: 1997-2003.
34. Baron, R., G. Rawadi and S. Roman, 2006. Wnt signaling: A key regulator of bone mass. Curr. Top. Dev. Biol., 76: 103-127.
35. Mani, A., J. Radhakrishnan, H.E. Wang, A. Mani and M.A. Mani *et al*., 2007. LRP6 mutation in a family with early coronary disease and metabolic risk factors. Science, 315: 1278-1282.
36. Ghadiri-Anari, A., Z. Mortezaii-Shoroki, M. Modarresi and A. Dehghan, 2016. Association of lipid profile with bone mineral density in postmenopausal women in Yazd province. Int. J. Reprod. Biomed., 14: 597-602.