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Original Research Article

Association between serum ferritin level and bone mineral density in adult females of Dhaka city

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ABSTRACT

Background: Osteoporosis and osteopenia are among the alarming issues worldwide affecting people of all ages. Bone mass is influenced by several factors including iron. Iron deficiency is common in Bangladesh among the women of reproductive age group. So, iron depletion could have a detrimental impact on bone, resulting in episodes of fracture. The present study was carried out to assess the association of serum ferritin level and bone mineral density in adult females.
Methods: A cross sectional study was conducted in the Department of Physiology, Dhaka Medical College, Dhaka from July 2018 to June 2019. A total of 122 adult females of age ranging from 18-44 years were enrolled. Eligible subjects were divided into three groups on the basis of hemoglobin concentration. The serum ferritin level and bone mineral density were measured. For statistical analysis, One-way ANOVA followed by Bonferroni test and Pearson's correlation coefficient (r) were performed as applicable using SPSS for windows version 25.0.
Results: The mean (±SD) serum ferritin level and BMD T score of lumbar spine and femoral neck showed significant differences among the groups ($p<0.001$, $p=0.002$, $p=0.001$ respectively). We found positive correlation ($r±0.378$ and $r±0.353$ respectively) between serum ferritin level and BMD T score of both lumbar spine and femoral neck which was statistically significant ($p<0.001$).
Conclusions: Low serum ferritin level is positively associated with low bone mineral density among adult females of Dhaka city. Therefore, early detection and correction of iron deficiency could be an important preventive measure against the disruption of bone composition at various stages of life.

Keywords: BMD, Bone health, Reproductive age females, Serum Ferritin

INTRODUCTION

Bone is a hard connective tissue consisting of supporting structures or skeletons. It serves as a reservoir of minerals mainly calcium phosphate and calcium carbonate and organic matter in the form of protein fiber and type I collagen.[1,2] Bone mineral density reflects the strength and quality of a bone.[3] The strength of a bone depends on the bone quality and density.[2] Bone density is defined as the grams of calcium present per square cm of the bone.[4] The quality of a bone refers to the architecture of the bone,

its mineral content and presence of accumulated micro fracture. The point at which the bone has attained maximum strength and density at the end of its maturation is known as peak bone mass. The peak bone mass attains 90% of its strength and density by 18 years in girls and 20 years in boys. Normally women have lesser bone mass in comparison to men.[2] An individual who does not acquire the peak bone mass during adolescence have a greater chance of developing early osteoporosis.[4] Bone is a living tissue which undergoes continuous modification and any deviation in the bone mineral content from its normal level could increase the risk of fracture and disability.[3]

The remodeling of bone occurs gradually but it may remain asymptomatic for many years.[5] Bone mineral density (BMD) is measured by DXA (Dual energy X-ray Absorptiometry). It is considered the most acceptable method used worldwide which helps to predict the risk of fracture as well as helps in monitoring the bone density of patients undergoing treatment.[6] The results of DXA are expressed in T score. T score indicates the difference of patient's measured BMD with the ideal peak bone mass of a young healthy adult of matched gender and ethnic group.[7] Recent studies suggest that low serum ferritin level is a risk factor for osteoporosis.[8,9]

According to the National Micronutrient Survey 2011-2012, in the urban areas of Bangladesh they found the prevalence of anemia of 21.4% in non-pregnant and non-lactating (NPNL) women of age ranging from 15-49 years. The prevalence of iron deficiency (defined as serum ferritin level <15 ng/ml) and iron deficiency anemia (defined as haemoglobin <12.0 g/dl and serum ferritin<15 ng/ml) among NPNL women of urban areas were about 8.7% and 4.1% respectively.[10]

In Bangladesh iron deficiency is mostly attributed to poverty, lack of education, chronic infection like hookworm infestation and chronic blood loss during menstruation.[11] The quality of food that is consumed is poor due to deficiency of micronutrients, more intake of cereal staples which contain high content of phytate that inhibits iron absorption.[12] The daily recommended allowance for iron is 15-18 mg/day in women, but in Bangladesh the total iron consumption among females is only 6.64 mg/day.[13] The major portion of iron that is consumed are from non-animal source and its bioavailability is very poor.[10]

Iron is an essential element required by the body for cell growth and functioning.[14] It is stored in the liver, spleen and bone marrow in the form of ferritin which is a non-toxic intracellular protein.[15] Serum ferritin is the most suitable indicator for estimating the iron stored in the body.[16] Iron is also involved in bone metabolism. It acts as an essential cofactor for the modification of procollagen into collagen by the addition of hydroxyl group to proline and lysine residue of the procollagen.[16,17] Availability of iron is also important for the metabolism of vitamin D which is regulated through cytochrome P450 enzyme.[16] The cytochrome P450 family consists of heme containing monooxygenases as the prosthetic group.[17,18] Different researchers and organizations of different countries have performed studies to assess the relation between serum ferritin level and bone mineral density among population of all age groups. However, in Bangladesh, there is less published data available regarding this topic. So the present study is designed to find the association between serum ferritin levels with bone mineral density among adult females of Dhaka city. The findings of the study may be useful in reducing the incidence of osteoporosis, especially in premenopausal women, thereby helping to prevent avoidable fractures and its associated socioeconomic burden.

METHODS

Study type

This was a cross-sectional study.

Study place

The study was conducted in the Department of Physiology, Dhaka Medical College, Dhaka, Bangladesh.

Study duration

The study period was from July 2018 to June 2019.

The research work was carried out after obtaining ethical clearance from the concerned Departments, Research Review Committee and Ethical Review Committee of Dhaka Medical College, Dhaka. The subjects were selected randomly from different areas of Dhaka city and were contacted either by phone or in person and were encouraged to take part in the study. Those subjects interested were contacted further and the details of the study procedure, its purpose and benefits were explained in very easy and understandable language. A total of 122 adult females were included in this study.

Inclusion criteria

Subjects were female of age ranging from 18 to 44 years; Bengali ethnicity; women with normal serum calcium level (8.20–10.20 mg/dl); normal body mass index (WHO 2000).[19]

Exclusion criteria

Subject with history of chronic liver disease, chronic renal disease, chronic inflammatory disease, diabetes, hypertension, thyroid disease, hemorrhagic disorder, hemoglobinopathies, any bone disease, malignancy, trauma. Subject with history of acute infection, acute hemorrhage. Blood donor. History of taking medications like vitamin D, calcium, iron, long time use of steroid (5 mg/day for last 3month), anticonvulsant, thiazide diuretics. Pregnancy, lactation.

Data collection

Prior to blood sample collection, informed written consent was taken from the participants. The subjects were interviewed in details regarding their personal, socioeconomic status, dietary, medical, drug, family history and history of physical activity. The subjects' height and weight were measured and body mass index was calculated. The pulse rate and blood pressure were measured.

All the information was recorded in a prefixed questionnaire. The confidentiality was maintained. Then with all aseptic precautions, 7 ml of venous blood was collected from antecubital vein by 10 cc disposable plastic syringe from each subject. The blood sample was transferred in 3 separate vacuum tubes i.e. 2 ml of blood was taken in a tube with EDTA anticoagulant and mixed well for the estimation of complete blood count (Hemoglobin concentration was measured by spectrophotometry method in (Horiba Pentra DX Nexus) automated haematology analyzer and total count of WBC was measured by impedence method in automated haematology analyzer).

Another 2 ml of blood was taken in a glucose tube containing sodium fluoride anticoagulant and mixed well for the estimation of random blood glucose and which was measured by hexokinase method in automated biochemistry analyzer. The rest 3 ml of blood was taken in a separate tube for biochemical tests.

All the blood samples were sent to Department of Laboratory Medicine, Dhaka Medical College Hospital. For the separation of serum, 3 ml blood tubes were centrifuged at a rate of 3000-4000 rpm for 10-15 minutes. After that, the supernatant serum was collected and analyzed for serum ferritin level and for exclusion criteria investigations (serum calcium, SGPT, serum creatinine).

Serum ferritin level was measured by enzyme labeled immunoassay using FERR method in Dimension xpand plus Automated Biochemistry Analyzer. Serum calcium was measured by calcium method in Automated Biochemistry Analyzer. Serum creatinine was measured by CRE2 method in automated biochemistry analyzer and serum SGPT was measured by Dimension ALTI method in automated analyzer machine.

The subjects were then placed into three groups (Group A, B, C) on the basis of haemoglobin concentration.[20] Each group consisted of a minimum of 35 subjects. Blood sample collection was done until the accomplishment of the minimum sample size in each group, so equal number of subjects could not be taken in each group.

A total of 127 blood samples were collected. Among them, 5 subjects were excluded due to higher total count of WBC or higher serum SGPT or RBS levels. After the exclusion, Group A, Group B, Group C included 47, 40 and 35 subjects respectively.

Subjects who fulfilled the inclusion and exclusion criteria of the study were advised for BMD measurement using QDR-2000 (Hologic, USA) based on the principle of Dual Energy X-ray Absorptiometry (DEXA) method which was done in the Institute of Nuclear Medicine and Allied Sciences (INMAS), Dhaka Medical College.

Statistical analysis

Data collected were processed by using a computer based statistical program SPSS (Statistical Package for Social Sciences) version 25.0. The parameters were expressed as mean and standard deviation (mean±SD).

One way ANOVA followed by Bonferroni test was performed to compare between the groups. Pearson's correlation coefficient (r) test was done to explore the association between serum ferritin levels with BMD. A difference was considered as statistically significant if p value <0.05.

RESULTS

Mean (±SD) of age, BMI, systolic and diastolic blood pressure were shown in Table 1. The mean (±SD) serum ferritin level of group A, group B and group C were 20.91±11.45, 45.90±16.69, 56.91±15.95 ng/ml respectively.

The mean (±SD) serum ferritin showed significant differences among the groups (p<0.001) Table 2 and Figure 1. The mean (±SD) serum ferritin level of subjects in group A was lower than those in group B and C which was statistically significant (p<0.001).

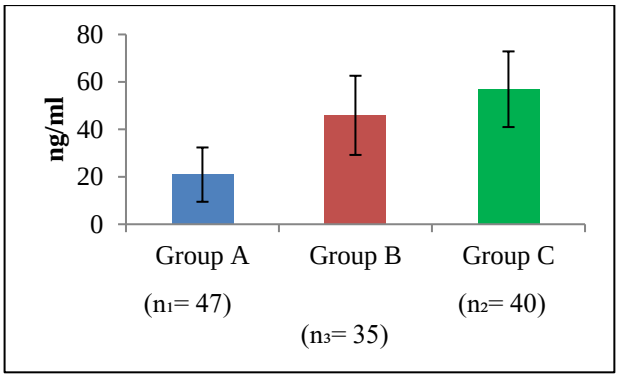

Figure 1: Mean serum ferritin level of the subjects in different groups (n=122).

Results are expressed as mean±SD**,** n=total number of subject, n1=number of subjects in group A, n2=number of subjects in group B, n3=number of subjects in group C**,** Group A: Hemoglobin concentration 8–10.9 gm/dl**,** Group B: Hemoglobin concentration 11–11.9 gm/dl**,** Group C: Hemoglobin concentration ≥12 gm/dl.

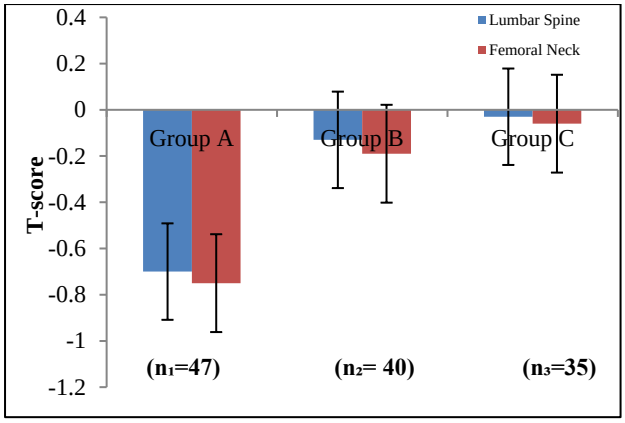

Figure 2: Mean BMD T score of the subjects in different groups (n=122).

Results are expressed as mean±SD, n=total number of subject, n1=number of subjects in group A, n2=number of subjects in group B, n3=number of subjects in group C; BMD–bone mineral density, Group A: Hemoglobin concentration 8–10.9 gm/dl, Group B: Hemoglobin concentration 11–11.9 gm/dl, Group C: Hemoglobin concentration ≥12 gm/dl.

The mean (±SD) serum ferritin level of subjects in group B was lower than those in group C which was also statistically significant (p=0.004). The mean (±SD) BMD T score of lumbar spine of group A, B and C were -0.70±1.01, -0.13±0.85, -0.03±0.92 respectively Table 2. The mean (±SD) BMD T score showed significant differences among the groups (p=0.002) Figure 2.

Table 1: General characteristics of the study subjects (n=122).

Parameters	Mean±SD
Age (in years)	28.09±4.713 (20-40)
BMI (kg/m^2)	23.77±1.35 (20.7–24.9)
Systolic pressure (mmHg)	113.28±12.18 (90–130)
Diastolic pressure (mmHg)	70.78±9.05 (60-85)

Results were expressed as mean ± SD. Figures in parenthesis indicate range, N- total number of subjects, BMI-body mass index.

The mean (±SD) BMD T score of subjects in group A was lower than those in group B and group C which was statistically significant (p=0.015 and p=0.005 respectively). The mean (±SD) BMD T score of subjects in group B was lower than those in group C which was not statistically significant (p=0.889). The mean (±SD) BMD T score of femoral spine of group A, B and C were -0.75±0.95, -0.19±0.85, -0.06±0.84 respectively Table II. The mean (±SD) BMD T score showed significant differences among the groups (p=0.001) Figure 2. The mean (±SD) BMD T score of subjects in group A was lower than those in group B and group C which was statistically significant (p=0.011 and p=0.002 respectively). The mean (±SD) BMD T score of subjects in group B was lower than those in group C which was not statistically significant (p=0.802). The results showed positive correlation which was statistically significant between serum ferritin level and BMD T score in lumbar spine (r±0.284, p=0.001) and BMD T score in femoral spine (r±0.366, p<0.001) Table III and Figure 3.

Table 2: Study parameters of the subjects in different groups (n=122).

	Groups		
Parameters	**A (n1=47)**	**B (n2=40)**	**C (n3=35)**
Ferritin (ng/ml)	20.91 ±11.45	45.90 ±16.69	56.91 ±15.95
BMD (T score)			
Lumbar spine	-0.70±1.01	-0.13±0.85	-0.03±0.92
Femoral neck	-0.75±0.95	-0.19±0.85	-0.06±0.84

Results are expressed as mean ± SD, N=total number of subjects, n=number of subjects in group A, n2=number of subjects in group B, n3=number of subjects in group C, BMD–bone mineral density, Hb–hemoglobin, Group A: Hemoglobin concentration 8–10.9 gm/dl, Group B: Hemoglobin concentration 11–11.9gm/dl Group C: Hemoglobin concentration≥12 gm/dl.

Table 3: Statistical analysis.

Groups	P value		
	Ferritin	BMD (Tscore)	
		Lumbar spine	Femoral neck
AvsBvsC	<0.001***	0.002***	0.001***
AvsB	<0.001***	0.015*	0.011*
AvsC	<0.001***	0.005**	0.002***
B vsC	0.004**	0.889ns	0.802ns

One way ANOVA followed by Bonferroni test was performed to compare between groups, The test of significance was calculated and *p* value<0.05 was accepted as level of significance, ns= not significant, */**/***=significant, BMD–bone mineral density, Hb–hemoglobin, Group A: Hemoglobin concentration 8–10.9 gm/dl, Group B: Hemoglobin concentration 11–11.9gm/dl, Group C: Hemoglobin concentration ≥12 gm/dl.

Table 3: Association of serum ferritin level with BMD T score in study subjects (n=122).

Parameters		r value	P value
Serum ferritin with	Lumbar spine	+0.378	<0.001***
	Femoral neck	+0.353	<0.001***

Pearson's correlation coefficient (r) test was performed to observe association between serum ferritin and BMD T score. The test of significance was calculated and p value<0.05 was accepted as level of significance***=significant; N=total number of subjects; BMD-bone mineral density.

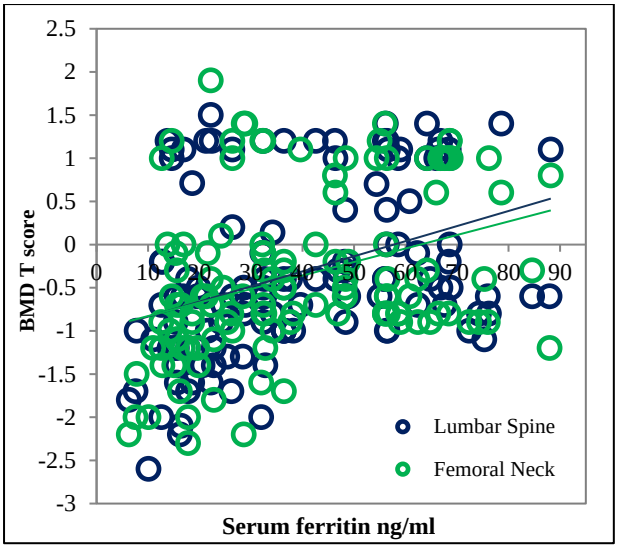

Figure 3: Association of serum ferritin level with BMD T score in study subjects (n=122).

BMD–bone mineral density, n=number of study subjects, Study subjects: Bangladeshi adult females, S. ferritin vs Lumbar spine, r±0.378, $p<0.001$***, S. ferritin vs Femoral neck, r±0.353, $p\leq0.001$***

DISCUSSION

The present study was undertaken to observe the association between serum ferritin levels with bone mineral density among adult females residing in different areas of Dhaka city. The subjects were assembled into three groups according to their hemoglobin concentrations, after taking detailed history to fulfill the inclusion and exclusion criteria of the study. The study showed, mean serum ferritin level of the subjects of all the groups were within normal range but closer to the lower limit. The mean differences among the groups were statistically significant (p<0.001).

The low serum ferritin levels among most of the subjects may be due to the dietary habit of the Bangladeshi people. They mostly consume rice and other cereal based diets like lentils, wheat and beans; fiber-rich leafy vegetables and fruits and meat occupies a very small portion of their meal. The leafy vegetables and fruits contain non-haem iron, which are less absorbed in the gastrointestinal tract than haem iron present in meat, liver etc. The plant based food and rice are also rich in polyphenols and phytates which inhibit iron absorption. Most of the subjects consumes about 2-6 eggs weekly.

The egg yolk contains phosvitin which is a potent inhibitor of iron absorption. Vitamin C consumption was also not adequate in quantity among the subjects. Our cooking methods may have a great impact in degrading the vitamin C and other nutrients present in the food. A decrease in the availability of vitamin C may have also reduced the absorption of non-haem iron from the food. All these contributed to decrease in the serum ferritin level among the subjects.

The mean BMD T score of both lumbar spine and femoral neck was found lower in group A than group B and group C. The mean differences among the groups for both lumbar spine and femoral neck were statistically significant (p<0.002 and p<0.001 respectively). Laudisio et al, Oraibi et al, also found lower BMD T score among the subjects with low hemoglobin levels than the subjects with normal hemoglobin levels.[21,22] Serum ferritin levels showed positive association with BMD T score both in the lumbar spine and femoral neck in the present study and was statistically significant (p<0.001). This association was observed may be due to decrease in synthesis of collagen and decrease in activation of vitamin D. Bone consists of 90% of type I collagen and these collagen fibers undergo posttranslational modification for its functional maturation.

The posttranslational modification occurs by the addition of hydroxyl group to the proline and lysine amino acid residue of the nascent peptide chain for the formation of triple helix.[23] This hydroxylation reaction is catalysed by prolyl and lysyl hydroxylase enzymes in which ferrous iron is an essential cofactor. Less availability of iron will cause disruption of cross linkage of the collagen and thus decrease the mineralization and the integrity of the bone.[24]

Vitamin D helps in bone mineralization and the active form of vitamin D (1, 25-dihydroxycholecalciferol) is formed in the kidney following the hydroxylation of 25(OH) D in the presence of 1α hydroxylase enzyme.[25] This enzyme comprises of three components involving cytochromes P-450, renal ferredoxin (iron-sulphur protein) and ferredoxin reductase.[26] The cytochrome P-450 is a heme containing protein which contains iron in its structure.[27] This iron activates the two important enymes (δ–aminolevulinic acid synthetase and ferrochelatase) which is needed for heme synthesis. Thus, less availability of iron may compromise the production of active form of vitamin D.[28]

In agreement with this study finding, similar type of observation was found by other researchers.[16,29,30] Jung et al, found no association in their study.

The study had potential limitations. Due to time constraint cohort study over a longer period could not be done. The study could have been done on a larger sample size involving other geographical areas of Dhaka city. The study was conducted only on subjects belonging to the middle class socioeconomic group which does not represent the entire socioeconomic group of Dhaka city. Also due to financial restrictions serum vitamin D and other parameters of iron profile could not be done.

CONCLUSION

After analysing the results of the study, it can be concluded that low serum ferritin level is positively associated with low bone mineral density among the adult females of Dhaka city. Therefore, early detection of iron deficiency

may be helpful for the maintenance of bone health in this group of females. Thereby, preventing the episodes of fracture and its associated socioeconomic burden.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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