ORIGINAL ARTICLE

https://doi.org/10.3329/jmcwh.v20i1.77146

ASSOCIATION OF HEMOGLOBIN CONCENTRATION WITH BONE MINERAL DENSITY IN ADULT FEMALES OF DHAKA CITY

Nishat Rahman[1*], Qazi Shamima Akhter[2], Manasi Saha[3], Khadijatul Busra[4], Mahbuba Akter[5], Nawshin Islam[6], Keya Sarker[6], Mostafizur Rahman[7], Nusrat Jahan[8].

ABSTRACT

Background: Globally osteoporosis is a major public health concern. Various factors have influence on bone mass. Anemia is common in Bangladesh among the women of reproductive age group. This may be a contributing factor to low density of mineral of bone in young adult females. **Aim:** This research was performed to observe hemoglobin concentration's association with density of bone mineral in adult females of Dhaka city. **Materials and Method:** This cross sectional study was performed in the Physiology Department of Dhaka Medical College, Dhaka between 2018 July and 2019 June. One hundred and twenty two female participants in the age range from 18 to 44 years were recruited for the study. The density of mineral of bone measurement was carried out for the eligible subjects after obtaining informed written consent. Data was collected and statistical analysis was done using One-way ANOVA, Bonferroni test and Pearson's correlation coefficient (r) using SPSS for windows version 25.0. **Results:** The mean (± SD) hemoglobin concentration of group A, group B and group C were 9.87 ± 0.71, 11.44 ± 0.26, and 12.75 ± 0.75 g/dl respectively. The mean (± SD) bone mass density (BMD) T score of lumbar spine of group A, group B and group C were -0.70 ± 1.01, -0.13 ± 0.85, and -0.03 ± 0.92 respectively. The mean (± SD) BMD T score of femoral neck of group A, group B and group C were -0.75 ± 0.95, -0.19 ± 0.85, and -0.06 ± 0.84 respectively. We found statistically significant positive correlation (r= +0.284 and r= +0.366 respectively) between BMD T score of both lumbar spine and femoral neck with hemoglobin concentration (p=0.001 and$p$<0.001 respectively). **Conclusion:** A reduction in hemoglobin concentration may lead to reduced oxygen in blood with stimulation of proteosomal degradation; osteoclastogenesis and inhibition of osteoblastic bone formation. These changes may cause decrease in bone mineral density. Therefore, early detection of anemia may help prevent reduction in density of bone mineral and maintain bone health.

Keywords: Hemoglobin, BMD, Bone health, Hypoxia

Cite this article: Rahman N, Akhter QS, Saha M, BusraK, AkterM, IslamN, SarkerK, RahmanM, Jahan N. Association of hemoglobin concentration with bone mineral density in adult females of Dhaka city. J Med Coll Women Hosp.2024;20(1):23-31

INTRODUCTION

Osteoporosis and osteopenia are the two public health problems characterized by low bone mass and density[1].Bone loss is a natural phenomenon of aging process and occurs in both genders[2]. An average of 0.7% of bone loss occurs normally per year[3].Lifetime loss may cumulate to 30-40% in women and 20-30% in men[4].The bone loss usually occurs more in women after menopause due to cessation of release of estrogen hormone. But nowadays, it is observed that the bone density is decreasing much earlier before menopause[1].

1*. Department of Physiology, Z.H. Sikder Women's Medical College, Dhaka. Bangladesh. Email: ive.got.mailtoo@gmail.com [Address of correspondence]
2. Department of Physiology, Dhaka Medical College, Dhaka, Bangladesh
3. Department of Physiology, Shaheed Ziaur Rahman Medical College, Bogura. Bangladesh.
4. Department of Physiology, Jashore Medical College, Jashore, Bangladesh.
5. Department of Physiology, Shaheed Tajuddin Ahmad Medical College, Dhaka, Bangladesh.
6. Department of Physiology, Mugda Medical College, Dhaka, Bangladesh.
7. Department of Physiology, Shaheed Syed Nazrul Islam Medical College, Kishorgonj, Bangladesh.
8. Department of Physiology, Bashundhara Addin Medical College, Dhaka, Bangladesh.

A group of researchers did a cross sectional study on 500 women aged 16-65 years. They found the prevalence of osteopenia and osteoporosis was 43.6% and 5.5% among 16-45 years old women and 40.7% and 41.8% among 46-65 years old women. The study advised that screening program should be taken up in reproductive and middle-aged women which would help lower the occurrence of fractures[5]. Any deviation in the normal level of BMD could increase the risk of fracture and disability[6]. BMD is measured by a method that is considered as 'Gold standard' known as DXA scan (Dual energy X-ray Absorptiometry)[7]. Low hemoglobin concentration have been suggested to be a factor for aggravating osteoporosis in previous research works[8,9]. Hemoglobin is a tetrametric structure formed by pairing of α and β globin chains. Majority of the adult hemoglobin is α_2 β_2 form[10].Hemoglobin transports oxygen from the lungs to the cells of the body and also transports metabolically produced carbon dioxide and hydrogen ion from the body to the lungs[11]. Oxygen is one of the regulators of osteoclastic activity in bone resorption[12]. A change of oxygen availability is regulated by a transcription factor called hypoxia inducible factor (HIF-1). It is a heterodimer composed of an alpha subunit and a beta subunit. At normal partial pressure of oxygen, the prolyl-4-hydroxylase enzyme hydroxylates a proline residue to α subunit of HIF-1. Thus facilitating its degradation in the proteosomes and preventing its further action[13]. However, in hypoxic condition, the decreased oxygen level reduces the activity of prolyl-4-hydroxylase enzyme that does not degrade HIF-1α. HIF-1 then enters the nucleus and increases transcription of erythropoietin (EPO) gene[14]. Erythropoietin stimulates the precursors of osteoclast cells and enhances bone loss[15]. Hypoxia also aggravates extracellular acidification as a result of anaerobic metabolism which activates the osteoclast cells and accelerates bone resorption[12].

Relationship between concentration of hemoglobin and density of bone minerals among population have been reported by various studies in different countries[5,6,8,9]. However, in Bangladesh, research work on this topic is scarce. The present study is designed to find the relationship between hemoglobin concentration and density of bone mineral in adult females of Dhaka city.

MATERIALS AND METHOD

Study design

In this study, cross sectional study design was carried out.

Study place and period

The place of study was Physiology Department, Dhaka Medical College, Dhaka, Bangladesh from 2018 July to 2019 June.

Study Population

The study population consisted of female individuals in the age group of 18-44 years.

Recruitment of participants

The procedure, benefits and purpose of the research was explained in details before the participants were included in the study. Before being recruited, the individuals signed an informed written consent.

Criteria of selection

Included in this research were female subjects with age range of 18-44 years withserum calcium level in normal range (8.20-10.20 mg/dl) and normal Body Mass Index (BMI).

Subjects with history of chronic inflammatory disease, chronic disease of liver, chronic kidney disease, thyroid disease, diabetes mellitus,hemorrhagic disorder, hemoglobinopathies, hypertension, any bone disease, malignancy, trauma were excluded from the study. Also excluded were those having history of acute infection,acute hemorrhage, taking medications like vitamin D, calcium, iron, long time use of steroid (5mg/day for last 3 months), anticonvulsant, thiazide diuretics.

Pregnant, lactating mothers and blood donors were not recruited in this research.

Sampling technique

Simple random sampling was performed.

Data collection

The recruits 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 BMI was calculated. Recording ofthe pulse rate and blood pressure were done. Information was recorded in a questionnaire that was prefixed.

Sample collection and analysis

With all aseptic precautions, 7ml of venous blood was collected from antecubital vein of the participants. The sample of blood was transferred in 3 separate vacuum tubes, that is, 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 Hematology Analyzer and total count of WBC was measured by impedance method in Automated Hematology 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 3ml of blood was taken in a separate tube for biochemical tests. All the blood samples were sent to Laboratory Medicine Department, 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 exclusion criteria investigations (serum calcium, SGPT, serum creatinine). 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 hemoglobin concentration[16].Each group consisted of a minimum of 35 subjects. Blood sample collection was done until the accomplishment of the minimum sample size. 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 or lower serum calcium level.

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.

Ethical approval

The research work was carried out after obtaining ethical clearance from the Research Review Committee and Ethical Review Committee of Dhaka Medical College, Dhaka.

Statistical analysis plan

SPSS (Statistical Package for Social Sciences) version 25 was used for statistical analysis. The results were expressed as mean and standard deviation (mean ± SD). For comparison between the groups One way ANOVA and after that Bonferroni test was done. Pearson's correlation coefficient (r) test was done to explore the association between hemoglobin concentrations with BMD. A difference was considered as statistically significant if

Hemoglobin concentration and bone mineral density

RESULTS

Displayed in Table 1 are the mean (±SD) of age, BMI, systolic and diastolic blood pressure.The mean (±SD) hemoglobin concentrations showed significant differences (p<0.001) among the groups as shown in Table 2 and Figure 1. The mean (±SD) hemoglobin concentration in Group A was significantly lower than Group B and group C (p< 0.001). The mean (± SD) hemoglobin concentration of Group B was lower than Group C which was also statistically significant (p<0.001).The mean (± SD) of BMD T score of lumbar spine of Group A, B and C were -0.70 ± 1.01, - 0.13 ± 0.85, and -0.03 ± 0.92 respectively. It showed significant differences among the groups (p =0.002). Table 2 shows the mean (± SD) BMD T score of femoral spine of Group A, B and C were -0.75 ± 0.95, - 0.19 ± 0.85, and -0.06 ± 0.84 respectively. It showed significant differences among the groups (p =0.001). The mean (± SD) BMD T score of femoral spine of Group A was lower than Group B and Group C which was statistically significant (p =0.011 and p =0.002 respectively). The mean (± SD) BMD T score of femoral spine of Group B was lower than Group C which was not statistically significant (p =0.802). Figure 2 illustrates the mean (± SD) BMD T score of lumbar spine of Group A was significantly lower than Group B and Group C (p =0.015 and p =0.005 respectively). The mean (± SD) BMD T score of lumbar spine of Group B was lower than Group C which was not significant (p =0.889). In Table 3 and Figure 3 the results showed statistically significant positive correlation between hemoglobin concentration 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 3 and Figure 3.

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

Parameters	Mean±SD
Age (years)	28.09±4.713 (20-40)
BMI	23.77 ± 1.35 (20.7–24.9)
Systolic pressure (mmHg)	113.28 ± 12.18 (90–130)
Diastolic pressure	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.

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

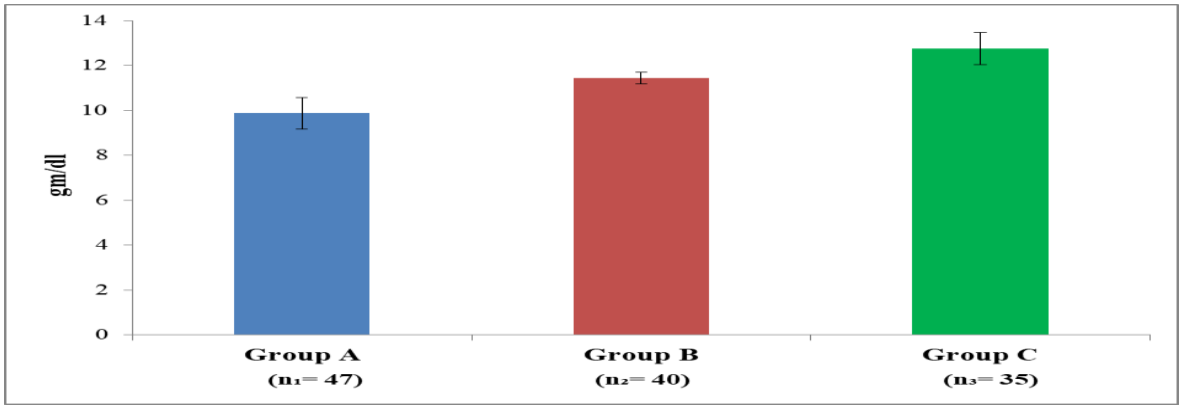
Parameters	Groups		
	A (n_1=47)	B (n_2=40)	C (n_3=35)
Hb (g/dl)	9.87 ±0.71	11.44 ± 0.26	12.75±0.71
BMD (T score)			
Lumbar spine	-0.70 ±1.01	-0.13 ±0.85	-0.03 ±0.92
Femoral spine	-0.75 ± 0.95	-0.19 ± 0.85	-0.06 ± 0.84

Results are expressed as mean ± SD ;N = total number of subjects; n_1 = number of subjects in group A; n_2 = number of subjects in group B; n_3 = 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 – 11.9 gm/dl; Group C: Hemoglobin concentration ≥ 12 gm/dl.

Groups	*P* Value		
	Hb	BMD	(T score)
A vs B vs C	<0.001***	0.002***	0.001***
A vs B	<0.001***	0.015*	0.011*
A vs C	<0.001***	0.005**	0.002***
B vs C	<0.001***	0.889ns	0.802ns

One way ANOVA followed by Bonferroni test was performed to compare between groups; *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 – 11.9 gm/dl ;Group C: Hemoglobin concentration= ≥ 12 gm/dl

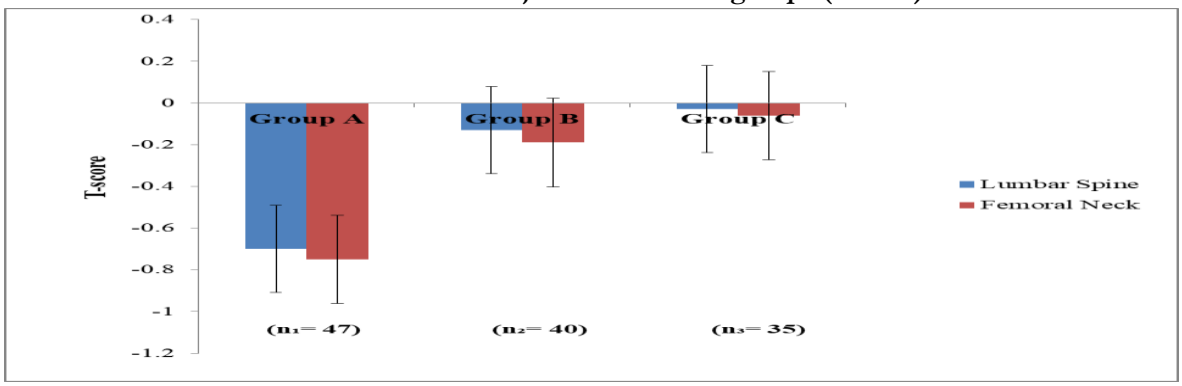
Figure-1
Mean hemoglobin concentration of the subjects in different groups (N=122)



Results are expressed as mean ± SD

N=total number of subjects; n_1=number of subjects in group A; n_2=number of subjects in group B; n_3= number of subjects in group C
Group A: Hemoglobin concentration 8 – 10.9 gm/dl
Group B: Hemoglobin concentration 11–11.9gm/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: n_1=numberofsubjectsingroupA; n_2=numberofsubjects ingroup B; n_3= number of subjects in group C
BMD= bone mineral density
Group A: Hemoglobin concentration 8 – 10.9 gm/dl
Group B: Hemoglobinconcentration11 -11.9gm/dl
Group C: Hemoglobinconcentration≥12 gm/dl

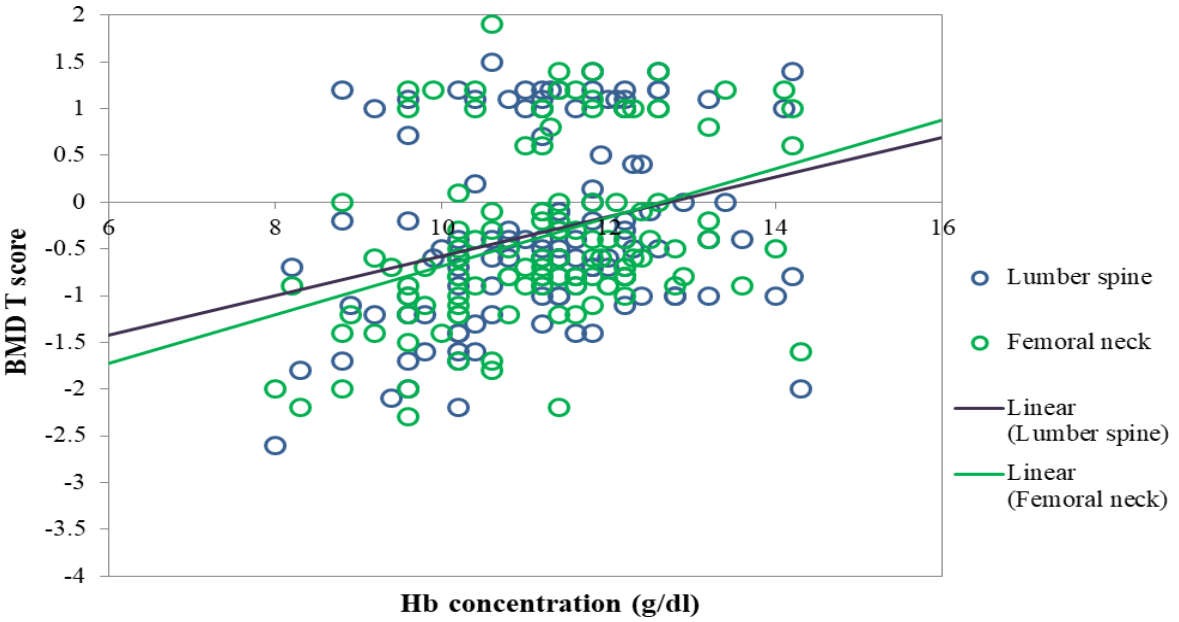
Table 3 : Correlation of hemoglobin concentration with BMD T score in study subjects (N=122)

Parameters	r value	*p* value
Lumbar spine Hbconcentration With Femoral neck	+ 0.284	0.001***
	+ 0.366	<0.001***

Pearson's correlation coefficient (r) test was performed to observe relationship between Hb concentration and BMD T score. The test of significance was calculated and *p* value<0.05wasacceptedas levelof significance;*** =significant; N=totalnumberofsubjects; Hb =hemoglobin;BMD=boneмineraldensity

Figure-3

Correlation of hemoglobin concentration with BMD T score in study subjects (N=122)



N = number of study subjects
Study subjects: Bangladeshi adult females

_____ Hbvs Lumbar spine
r =+0.284; *p*=0.001***

_____ HbvsFemoral neck
r = + 0.366; *p*= <0.001***
Hb=hemoglobin; BMD=bone mineraldensity

DISCUSSION

The present study showed, mean hemoglobin concentration of Group A was lower than Group B and C. Group B's concentration of hemoglobin was also lower than that of Group C. The mean differences among the groups were statistically significant ($p < 0.001$).

In this study, the concentration of hemoglobin was found lower among majority of the subjects. Inadequate dietary content of the Bangladeshi females may be a cause for the outcome of this study. The monthly menstrual bleeding could be another contributing factor. These may have caused the decrease in the hemoglobin concentration among the subjects.

The blood loss in women during menstruation creates a demand on the hematopoietic system. It does so by increasing the levels of hematopoietic growth factors and intensifying the proliferation of the hematopoietic progenitor cells, osteoblast and osteoclast cells, which causes expansion of the hematopoietic territories of the bone. The osteoclast cells cause bone resorption followed by release of bone morphogenic proteins which increase the production of osteoblast and further activation of osteoclasts. The combined activity of the blood cell production with expansion of the hematopoietic area may gradually lead to development of low bone density[17].

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). Hemoglobin concentration showed correlation that was positive with BMD T score both in the lumbar spine and femoral neck and the relation was statistically significant (p< 0.001).

A decrease in hemoglobin level reduces the oxygen supply to tissues and organs. In hypoxic condition, Hypoxia inducible factor (HIF) functions as the major transcriptional regulator. HIF-1α subunits have a specific function in the regulation of hypoxic inducible gene. It is an oxygen labile protein and is rapidly stabilized in hypoxia[18].In normal oxygen level, the proline residue of the HIF-1α undergoes hydroxylation in the presence of prolyl hydroxylase domain (PHD) enzymes. The activity of this enzyme requires α- 58 ketoglutarate, molecular oxygen and iron. This prolylhydroxylation of HIF1α thus promotes proteosomal degradation[13].

However, in hypoxic condition, the prolyl hydroxylase enzyme is inactive and the HIF-1α is not degraded. HIF1α enters the nucleus and binds with hypoxia response elements of DNA regulating EPO gene expression. Erythopoietin then mediates its function directly by binding to the EPO-R present on the osteoclast cells stimulating osteoclastogenesis and promoting bone resorption. It also inhibits the osteoblastic bone formation[15].

Similar studies were done by several researchers on anemic and non-anemic subjects and also showed positive correlation with BMD T score both in the lumbar spine and femoral neck and the relation was statistically significant (p <0.001)[8,19,20,21,22,23]. Some found no significant association between concentration of hemoglobin and BMD T score[9,24].

CONCLUSION

After analyzing the results of the study, it can be concluded that there is association between hemoglobin concentration and density of bone mineral among the adult females of Dhaka city. In this study, significant association was noted between lower hemoglobin concentration and lower density of bone mineral among the study groups. Therefore, early detection and correction of cause of anemia may be helpful for the maintenance of better bone health and thereby may play a significant part in the prevention of osteoporosis.

CONFLICT OF INTEREST

There is no conflict of interest

REFERENCES

1. ChadgalS, Talwar I. Prevalence of osteopenia and osteoporosis among young adult girls residing in the hostels of Panjab University, Chandigarh. Int J Academ Res Develop.2017;2(6):315-21.

2. Theintz G, Buchs B, Rizzoli R, Slosman D, Clavien H, SizonenkoPC,et al. Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J ClinEndocrinol Metabol,. 1992;75(4):1060-65

3. Malhotra N, Mithal A.2008. Osteoporosis in Indians. Indian JMed Res.2008;127(3):263-68

4. Peck WA, Burckhardt P, Christiansen C, Fleisch HA, Genant HK, GennariC,et al. Consensus Development Conference: diagnosis, prophylaxis, and treatment of osteoporosis.AmJ Med.1993;94(1):646-50

5. Begum RA, Ali L, Akter J, Takahashi O, Fukui T, RahmanM. Osteopenia and osteoporosis among 16-65 year old women attending outpatient clinics. J Community Health.2014;39(6):1071-76

6. Modagan P, Silambanan S, Menon PG, ArunalathaP.Comparison of bone mineral density with biochemical parameters and prevalence of osteopenia and osteoporosis in South Indian population. Biomed Pharmacol J.2018;11 (4): 2209-14

7. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak bone mass in the prevalence of osteoporosis. SaludPublica Mex. 2009; 51 Suppl 1:S5-17. doi: 10.1590 /s0036-36342009000700004.

8. Oraibi FA, Khan JA, Ardawi MS. Changes in hematological markers associated with bone turnover in male subjects. Int J Life Sci Res.2015; 3 (4):47-54.

9. Valderrábano RJ, Lee J, Lui LY, Hoffman AR, Cummings SR, Orwoll ES, et al. Osteoporotic Fractures in Men (MrOS) Study Research Group. Older Men With Anemia Have Increased Fracture Risk Independent of Bone Mineral Density. J ClinEndocrinolMetab. 2017; 102(7): 2199-2206. doi: 10.1210 / jc.2017-00266.

10. Farid Y, Bowman NS, Lecat P. Biochemistry, Hemoglobin Synthesis. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https: //www.ncbi.nlm.nih.gov/books/NBK536912/

11. Dissociation Curve. [Updated 2023 Mar 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/book/NBK539815/.

12. Arnett TR, Gibbons DC, Utting JC, Orriss IR, Hoebertz A, Rosendaal M, et al. Hypoxia is a major stimulator of osteoclast formation and bone resorption. J Cell Physiol. 2003; 196(1):2-8. doi: 10.1002/jcp.10321.

13. Gorres KL, Raines RT. Prolyl 4-hydroxylase. Crit Rev BiochemMol Biol. 2010; 45(2):106-24. doi: 10.3109/10409231003627991.

14. Haase VH. HIF-prolyl hydroxylases as therapeutic targets in erythropoiesis and iron metabolism. Hemodial Int. 2017; 21Suppl 1(Suppl 1):S110-S124. doi: 10.1111/hdi.12567.

15. Hiram-Bab S, Liron T, Deshet-Unger N, Mittelman M, Gassmann M, Rauner M, et al. Erythropoietin directly stimulates osteoclast precursors and induces bone loss. FASEB J. 2015; 29(5):1890-900. doi: 10.1096/fj.14-259085.

16. World Health Organization (WHO). Hemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011; p. 3. Accessed on May 12, 2019. Available from http://www.who.int/iris/handle/10665/85839.

17. Gurevitch O, Slavin S. The hematological etiology of osteoporosis. Med Hypotheses. 2006; 67(4):729-35. doi: 10.1016/j.mehy.2006.03.051.

18. Masson N, Ratcliffe PJ. HIF prolyl and asparaginyl hydroxylases in the biological response to intracellular O(2) levels. J Cell Sci. 2003;116(Pt 15):3041-9. doi: 10.1242/jcs.00655.

19. Yoon J, Kim D-J, Sung H-H, Jo Y-K., 2016. Analysis of bone mineral density according to hemoglobin in university students. Korean J Clin Lab Sci.2016; 48 (4):296-303.

20. Laudisio A, Marzetti E, Pagano F, Bernabei R, Zuccalà G. Haemoglobin levels are associated with bone mineral density in the elderly: a population-based study. ClinRheumatol. 2009; 28(2):145-51. doi: 10.1007/s10067-008-0998-6.

21. Korkmaz U, Korkmaz N, Yazici S, Erkan M, Baki AE, Yazici M, et al. Anemia as a risk factor for low bone mineral density in postmenopausal Turkish women. Eur J Intern Med. 2012; 23 (2): 154-8. doi: 10.1016/j.ejim.2011.11.009.

22. Cesari M, Pahor M, Lauretani F, Penninx BW, Bartali B, Russo R, et al. Bone density and hemoglobin levels in older persons: results from the InCHIANTI study. Osteoporos Int. 2005; 16(6):691-9. doi: 10.1007/s00198-004-1739-6.

23. Chuang MH, Chuang TL, Koo M, Wang YF. Low Hemoglobin Is Associated With Low Bone Mineral Density and High Risk of Bone Fracture in Male Adults: A Retrospective Medical Record Review Study. Am J Mens Health. 2019;13(3):1557988319850378. doi: 10.1177/1557988319850378.

24. Jørgensen L, Skjelbakken T, Løchen ML, Ahmed L, Bjørnerem A, JoakimsenR,et al. Anemia and the risk of non-vertebral fractures: the Tromsø Study. Osteoporos Int. 2010; 21(10):1761-8. doi: 10.1007/s00198-009-1131-7.