HEMOGLOBIN AND PLATELETS WITH BONE MINERAL DENSITY

Mário Maciel de Lima Júnior[1]
Maria da Conceição Lima Chaves[2]
Ronaldo da Silva Cruz[3]
Magna Bezerra Feitosa [4]

Resumo: Avaliamos a associação entre hemoglobina e plaquetas com densidade mineral óssea em homens entre 40 e 80 anos em Boa Vista, capital do estado de Roraima. O estudo populacional realizado neste estudo foi baseado no modelo de nomograma da Fracture Risk Assessment Tool proposto pela Organização Mundial da Saúde. Especificamente, o presente trabalho se concentra em (i) determinar a correlação inversa entre o escore de hemoglobina e fêmur e entre o escore de hemoglobina e coluna pelo nomograma FRAX® e usando o modelo de regressão linear, e (ii) correlacionar os fatores de risco com diferentes variáveis categóricas e variáveis contínuas usando métodos estatísticos. Este estudo de coorte e transversal envolve dados quantitativos e qualitativos obtidos em campo de 272 pacientes. No entanto, para este artigo, apenas os achados quantitativos da triagem são descritos. Além disso, poucos participantes participaram voluntariamente por demanda espontânea sendo encaminhado do Hospital Estadual Coronel Mota. Os dados foram coletados apenas conforme a diretriz do IBGE. A pontuação média de Frax e o nível de hemoglobina em todo o grupo foram significativamente diferentes. O escore Frax do fêmur foi significativamente associado à osteopenia e osteoporose da coluna. Todos os dados obtidos neste estudo foram analisados no SPSS versão 21, e um valor de p ≤0,05 foram considerados estatisticamente significativo. Hemoglobina e plaquetas estão fortemente associadas à DMO e os principais fatores de risco para a associação entre hemoglobina e plaquetas com DMO incluem IMC, tabagismo e etilismo, vitamina D e nível sérico de cálcio.

Palavras-chave: Hemoglogina; Osso; Densidade mineral

Abstract: We evaluated the association between hemoglobin and platelets with bone mineral density in men between 40 and 80 years of age in Boa Vista, the capital of the state of Roraima. The population study performed in this study was based on the Fracture Risk Assessment Tool nomogram model proposed by the World Health Organization. Specifically, the present work focuses on (i) determining the inverse correlation between the hemoglobin score and the femur and between the hemoglobin score and the spine by the FRAX® nomogram and using the linear regression model, and (ii) correlating the risk with different categorical variables and continuous variables using statistical methods. This cohort and the cross-sectional study involve quantitative and qualitative data obtained in the field from 272 patients. However, for this article, only quantitative screening findings are described. In addition, few participants participated voluntarily by spontaneous demand being referred from the Hospital Estadual Coronel Mota. Data were collected only by the IBGE guideline. The average Frax score and hemoglobin level across the group were significantly different. The Frax score of the femur was significantly associated with osteopenia and osteoporosis of the spine. All data obtained in this study were analyzed in SPSS version 21, and a p-value ≤0.05 was considered statistically significant. Hemoglobin and platelets are strongly associated with BMD and the main risk factors for the association between hemoglobin and platelets with BMD include BMI, smoking and alcohol consumption, vitamin D, and serum calcium level.

Keywords: Hemoglobin; Bone; Mineral Density

[1]Docente do Programa de Pós-graduação em Ciências da Saúde – PROCISA. e-mail: mmljr@uol.com.br
[2]Enfermeira, discente do Programa de Pós-graduação em Ciências da Saúde – PROCISA. e-mail: enfermeiraconceicaochaves@hotmail.com
[3]Fisioterapeuta, discente do Programa de Pós-graduação em Ciências da Saúde – PROCISA
[4]Discente no curso de Bacharelado em Enfermagem da FARES

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1.INTRODUCTION

BMD is acquired even before attaining adulthood (KOUDA et al, 2017). Several different components like osteoblasts, osteocytes, osteoclasts (or the remodeling cells), a non-mineral matrix of collagen, osteoid (non-collagenous proteins), and inorganic mineral salts make up the bone mass. Throughout the lifetime the entire bone mass network undergo repeated remodeling to protect the critical and vital organs of the body (BOSKEY e COLEMAN, 2010). Apart from protecting the vital organs, bones are the primary source of minerals, growth factors, cytokines, and home to calcium homeostasis (TAICHMAN, 2005).

Hemoglobin is an iron-containing metalloprotein that transports oxygen in the red blood cells of all vertebrates. Deficiency in these metalloprotein or red blood cells is a medical condition defined as Anemia. Osteoporosis is a skeletal disorder caused by compromised bone strength that eventually leads to an increased risk of fractures of different skeletal sites in the body (LANE, 2005).

While various genetic or constitutional factors are reported as the causal risk factors for osteoporosis, modifiable factors or the genetically determined factors play a pivotal role in the etiology of the disease (NEWS, 2001; SPALLARGUES et al.,2001; TANG et al., 2007). Other factors that contribute significantly to osteoporosis include lower body weight, age and dyslipidemia among many others and can be categorized into at least five different categories; genetics, lifestyle, chronic disease conditions, prior or current active medications, and others (NOF, 2008). On the same note, we would like to emphasize that adequate prevention is the key to controlling lower BMD. In one of their publications, the National Osteoporosis Foundation (NOF), emphasizes the requirements that cover a few daily lifestyle adaptations which include control of taking excess tobacco products (including alcohol), regular exercise, and adequate intake of vitamin D and calcium (NOF, 2008; FUGIMOTO, 1999; KARADAG, 2003) Even though our study includes only the male population, according to a survey conducted by the National Health Services in 2011, it is reported that the lack of enough red blood cells or the anemic condition is a typical disease state, prevalent in both men and women aged >12 years. During that survey, it was also found that the prevalence is more common in women (12.7%) than in men (2.4%). It was interesting to find that at old age (>70), the prevalence rate is almost

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equal in both men and women (~15% in men and ~18% in women). Regardless, anemia primed whit low hemoglobin levels itself is a known risk factor for many different kinds of diseases, e.g., Hypoxia is a risk factor for osteoporosis (FUGIMOTO et al., 1999; KARADAG et al., 2003; LAUDISIO et al., 2008).

Previously, a critical study has been undertaken to reveal the exact mechanisms of bone loss using mainly either blood samples or urine samples from patients with normal or low BMD (LIN, et al. 2006). That study was insightful in understanding not only understanding the possible causes of bone metabolism but also differentiating osteoporosis from other osteopenic conditions such as osteomalacia (which is considered a secondary cause of osteoporosis). According to Matkovic and collaborators (1992), factors that influence the formation and maintenance of bone includes but are not limited to different hormones, calcium levels, daily physical activities, prior history of medicine, and intoxications (MATKOVIC, 1992). Also, related but different reports suggest a possible association between bone density and hemoglobin levels in disease conditions such as sickle-cell anemia or chronic inflammatory conditions (SARRAI et al., 2007; GASCHE, 2000; TAAL et al., 1999). Irrespective of all the available information, the association between hemoglobin and platelets with BMD is elusive and needs more attention (CESARI et al., 2005; LAUDISIO et al., 2009). According to Cesari and collaborators (2005), levels do associates with hemoglobin level both in males and females as determined in a local Italian population. Also, a few previous pieces of literature also suggest a direct correlation between lower hemoglobin levels and bone mass density (TAAL et al.,1999; KENNY et al.,2003; FUJIMOTO et al., 1999; VICHINSKY et al.,1998). However, the study approach in these studies was complex, unconventional, and not targeted to study in mid-age to the old age group patient population. Apart from that, in these studies, even though the authors have established an association between the hemoglobin level and BMD, they fail to differentiate between the trabecular and cortical bones, two critical components of BMD. In this current study, we have evaluated the association between hemoglobin and platelets with BMD in men in an age range of 40 and 80, assessed by FRAX® nomogram.

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2. METHODS

Inclusion and Exclusion criteria: Male individuals in the age group between 40 years and 80 years old, and individuals who agree to participate in the research program and signed the free and informed consent form were included in the inclusion criteria. Female individuals males with any prior history of transplantation, or any sign of neurological disorder (stroke), autoimmune symptoms (e.g. lupus, inflammatory disease or intestinal disease), hematological disease (e.g. leukemia, myeloma, lymphoma), under the treatment of osteoporosis (lack of vitamin D or calcium), in the use of corticosteroids, in a process of hormone replacement, a carrier of either hypo or hyperthyroidism were included under exclusion criteria.

Study selection: Assuming 50% of the people with osteoporosis, with 5% error and 80% power, the required sample size, was estimated to be 380. The research participants were selected randomly. Also, few participants voluntarily participated upon spontaneous demand and were referred from the Coronel Mota state hospital.

Data Collection: Blood sample from each participant was collected by a group of the competent and trained laboratory team. The disposal of laboratory waste was done according to the SUS network. Collected blood samples were used to measure the serum concentration of calcium, vitamin D, hemoglobina, and platelet counts. Followed by, bone densitometry exam was done for each participant. Required data were collected to calculate the probability of fractures in the FRAX® nomogram. Clinical diagnosis of everyone was delivered and directed to the medical conduct by a medical professional of the Hospital Estadual Coronel Mota, as well as the necessary prescription or need for other tests. On the day of sample collection, participants have explained the study and procedure in the local language. Following this, informed consent was sought. Only, those who agreed to participate in the study and signed the informed consent form were considered for sample collection. Besides these, information on age, marital status, smoking, and alcohol habits were collected through a questionnaire. The height and weight of each participant were measured for the calculation of BMI. The tool was adapted from the WHO-FRAX® fracture risk assessment instrument. The Ethics Committee approved the project with a number 50207115.7.0000.5301. The data was collectedonly according to the guideline of IBGE (The Brazilian Institute of Geography and Statistics).

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Statistical Analysis: Data analysis was performed using STATA (STATA corp 14, Texas). Categorical variables are presented with frequency and percentage. Mean/median and their dispersion summarize continuous variables. For analysis purposes, variables are described across the FRAX femur score category (<3% no risk, >=3% at risk) as per the Sheffield cut off criteria. Independent t-test did the univariate analysis for continuous data and categorical data; the chi-square test was done. For non-normal data, the Mann-Whitney test was done to detect the significant difference. One last outlier information was removed from the analysis to make the distribution normal. A linear regression model was used to predict the association of hemoglobin with FRAX score femur and spine, separately. Multivariate regression analysis was done to estimate the adjusted coefficient hemoglobin after considering other variables. For all the analyses, statistical significance was decided at p-value <0.05.

3. RESULTS

Of the total, complete information was available for 272 participants. The mean age of the study participants was 58.38 years (SD 10.10) and was higher in the group having a hip Frax score ≥ 3. Details of the descriptive characteristics are provided in **table-1**.

Table-1: Descriptive statistics of continuous variables. *P value <0.05, ^Median and IQR, $ Mann Whitney test, for rest independent t-test.

Variables	**No risk (Frax<3) N=185 Mean/Median (SD/IQR)**	**At risk (Frax >=3) N=87 Mean/Median (SD/IQR)**	**Total N=272 Mean/Median (SD/IQR)**	**P value**
Age	57.56[9.79]	60.13[10.57]	58.38[10.10]	0.050
Height in meter	1.66[0.08]	1.67[0.08]	1.66[0.08]	0.596
Weight in Kg	74.85[13.36]	74.04[11.02]	74.59[12.64]	0.625
BMI m/kg^2	27.15[5.76]	26.53[3.40]	26.95[5.13]	0.358
Vitamin D ng/mL	43.23[10.68]	42.96[12.28]	43.15[11.20]	0.452
Serum calcium (mgdL)	8.92[1.02]	8.95[1.03]	8.93[1.02]	0.164
Haemoglobin g%	12.52[0.79]	17.95[0.21]	14.41[1.33]	<0.001*
Frax spine^	0.2[0.3]	1[1.2]	0.3[0.5]	<0.001*$
Platelets (mm3)^	211500[116000]	208000[125000]	209000[68000]	0.251$

Source: authors (2022).

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A significant difference in the mean score across the group was observed for the hemoglobin and Frax score of the spine at a p-value less than 0.05. The Frax score of the femur was significantly associated with osteopenia and osteoporosis of the spine, decided by T-Score and provided in **table-2**.

Table-2: Descriptive statistics of categorical variables

Variables	No risk (Frax<3) N=185	At risk (Frax >=3) N=87	Total N=272	Chi Square test P value
Osteopenia of spine	24.32[18.09-30.55]	40.22[29.82-50.64]	29.41[23.96-34.86]	0.007*
Osteoporosis of spine	7.56[3.72-11.40]	28.73[19.12-38.34]	14.33[10.14-18.52]	<0.001*
Marital status Single	28.64[22.08-35.21]	28.73[19.12-38.34]	28.67[23.27-34.08]	0.988
Married	71.36[64.78-77.91]	71.27[61.65-80.87]	71.32[65.91-76.73]	
Current smoke	5.94[2.51-9.37]	10.34[3.88-16.81]	7.35[4.23-10.47]	0.195
Current alcohol	23.78[17.60-29.96]	24.13[15.05-33.22]	23.89[18.79-28.99]	0.949

Source: authors (2022)

The inverse correlation of hemoglobin with Frax score of femur and spine are given in scatter plot distribution in Figures 1 and 2, respectively.

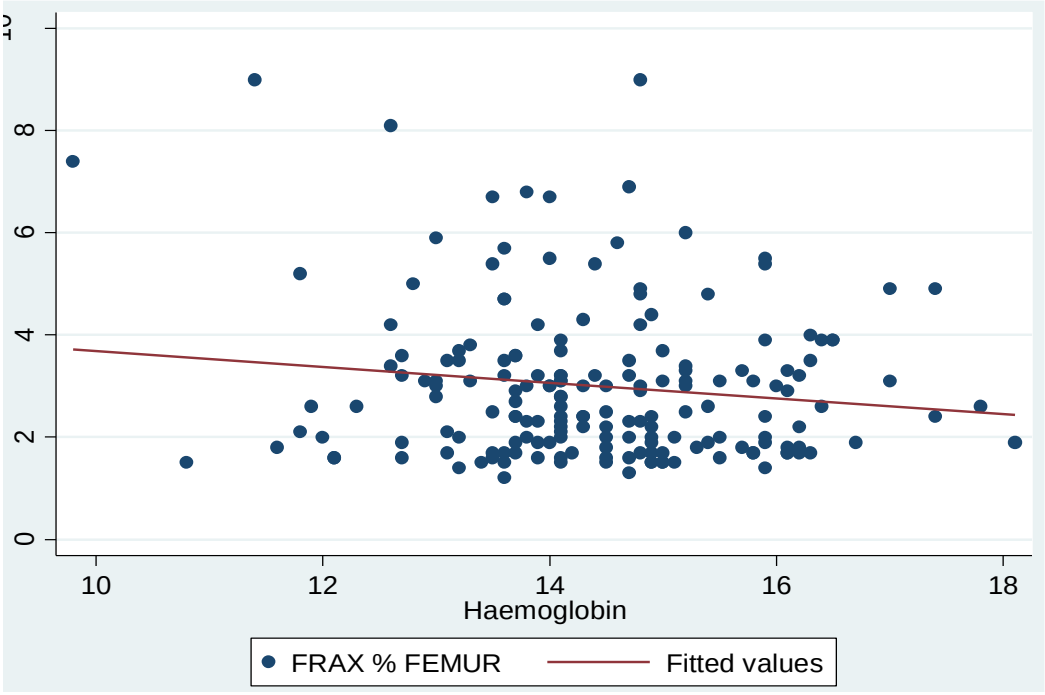

Figure -1: Scatter plot of Hemoglobin with Frax Femur score displaying the correlation between hemoglobin levels and the bone mineral density in study population (n=272).
Source: authors (2022)

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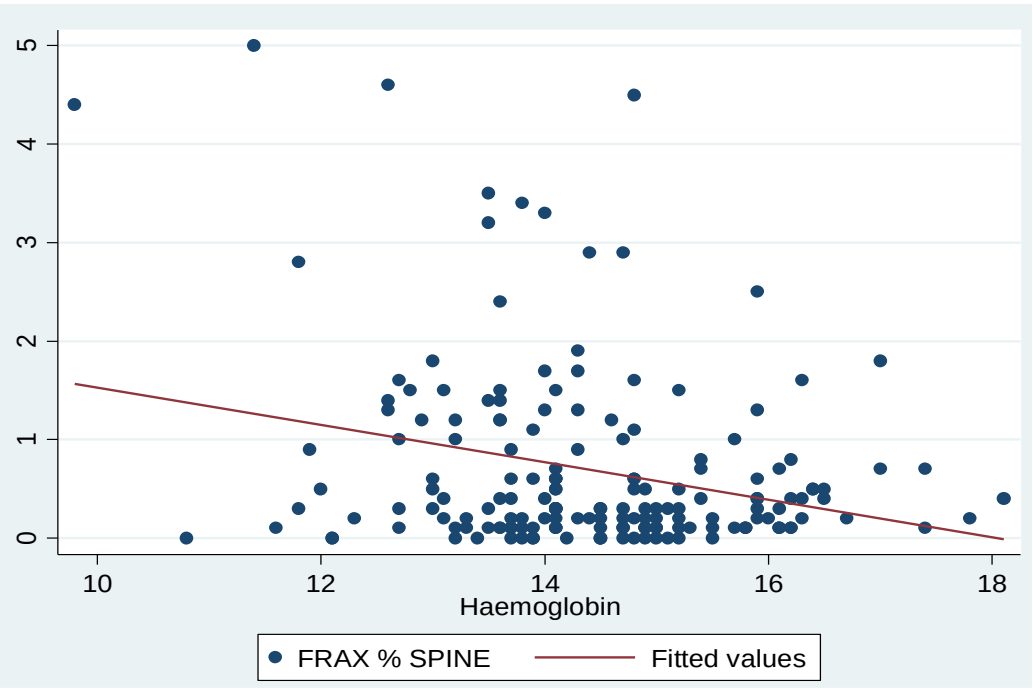

Figure -2: Scatter plot of Hemoglobin with Frax Spine score displaying the correlation between hemoglobin levels and the bone mineral density in study population (n=272).
Source: authors (2022)

We also performed two linear regression models, to predict the association of hemoglobin with the femur Frax score and spine Frax score, separately. The unadjusted regression coefficient for femur Frax score was -0.25 [95% CI: -0.45 to -0.04]. For every unit change in hemoglobin level (g%) there was a 0.22 units reduction in femur Frax score after adjusting for age, BMI, current smoking and alcohol habit, vitamin-D, and serum calcium level, which was statistically significant at the level p-value <0.05 and is provided in **table 3**.

Table 3: Linear regressions with femur FRAX score. *P value<0.05 # adjusted for BMI, Age, current smoking habit, alcohol habit, Vitamin-D level and serum calcium level.

Variables	Crude Coefficient [95% CI]	Adjusted Coefficient [95% CI]
Haemoglobin in g%	-0.25[-0.45 to -0.04]*	-0.22[-0.42 to -0.02]*#

Source: authors (2022)

A similar inversion relationship was seen with the spine Frax score, where the adjusted coefficient was -0.24 [95% CI -0.38 to -0.10] and tabulated in the **table – 4.**

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Table 4: Linear regressions with spine FRAX score. *P value<0.05 # adjusted for BMI, Age, current smoking habit, alcohol habit, Vitamin-D level and serum calcium level.

Variables	Crude Coefficient [95% CI]	Adjusted Coefficient [95% CI]
Haemoglobin in g%	-0.28 [-0.43 to -0.14]*	-0.24 [-0.38 to -0.10]

Source: authors (2022)

4. DISCUSSION

The current study evaluates the association between hemoglobin and platelets with BMD in men in an age range of 40 and 80 in the Boa Vista, the capital of the Brazilian state of Roraima using a FRAX® nomogram. In this study, compared to healthy patients, anemic patients had significantly lower bone mass density. However, those anemic patients were older than the control group. Besides, we observed a strong and independent association of hemoglobin with the femur Frax score and spine Frax score after controlling for BMI and other potential risk factors (smoking and alcohol habit, vitamin-D, and serum calcium level) in the linear regression analysis. The tight association of the hemoglobin levels to the BMD in our study is not eccentric. On fact, it is in agreement with several previous studies, where the prevalence of osteoporotic fractures is frequent in an aged population (KANNUS et al., 1999; Cooper et al., 1992; NCHS, 1992). In contrary, evidence from epidemiological data suggests that anemia is more frequent in an older population, and about more than 13% of the older population in the age range of 70-80 gets affected by this medical condition (NCHS, 1992). Not only that the anemic condition influences the olderpopulation it is also regarded as one of the dominant risk factors that lead to disability in the elderly (PENNINX et al.,2003; PENNINX et al.,2004; WOODMAN et al., 2005). However, we speculate that the defect in locomotor activity may be because of weak and poor muscular strength which isa secondary effect of reduced hemoglobin level (PENNINX et al.,2003; PENNINX et al.,2004; WOODMAN et al., 2005; KANIS and GLUER, 2000; MINEO et al, 2005).

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The medical conditions diagnosed in association with anemia may or may not directly influence the prevalence of osteoporosis (GASCHE, 2000; TALL et al., 1999; LENG, et al., 2002). However, it is possible that anemia may influence osteoporosis independently (CESARI et al., 2005). In this current work, our data suggest the following situation where the association between low hemoglobin level and BMD may be an independent event. The overall contribution of risk factors is also crucial. In corroboration with Laudisio (2007) we found that different confounding factors like BMI, smoking alcohol habit, vitamin-D, and serum calcium level influence the bone mineral density in the older subject significantly (CESARI et al., 2005). As described in the result section, we did utilize the power of linear regression analysis to minimize the effect of the confounders on the association between low hemoglobin level and BMD.

We observed that the overall association between the hemoglobin levels with BMD did not change even after establishing linear regressions with femur FRAX score (table 2) and with spine FRAX score (table 3), where the confounders are appropriately adjusted. This observation suggests a potential clinical and therapeutic intervention to low BMD, adjusting the hemoglobin level in patients. Regardless of any therapeutic intervention, our observation of an association between the hemoglobin levels with BMD will add additional information to the existing knowledge on different risk factors in the field.

Although our findings are in agreement with previous studies [18, 32], our data represents a strong association between BMD losses in general to that of hemoglobin levels and not to a specific bone. Besides, in corroboration with Laudisio and collaborators (2009), our data suggest that proper control of potential confounders is essential to meet the desired association between hemoglobin and BMD levels [18]. Fail to do so; patient population with related medication may contribute to the outliers and hence misrepresentation of the data. It is noteworthy that the findings from these two studies are restricted to a particular ethnic group and may not truly applicable to other ethnic groups.

The current study is cross-sectional, and hence it is hard to speculate the origin of lower BMD; whether before or after the development of anemia. So it did not allow us to confirm the cause-effect relationship. It would have been more impactful to gather information on potential treatment efforts where improving hemoglobin levels would improve the BMD quality. We also

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did not include a possible investigation to test if a hormone replacement option or calcium supplements would improve the BMD quality and leave us any clue about its association with hemoglobin level.

In conclusion, our study indicates that the key risk factors for the association between hemoglobin and platelets with bone mineral density include BMI, smoking and alcohol habit, vitamin-D, and serum calcium level. Our study also suggeststhat the hemoglobin and platelet levels in the given population are strongly associated with the BMD, once these confounders are tightly controlled. However, further clinical investigations are required to gain a better understanding of the overall pathophysiological pathways that connect the hemoglobin level and BMD in patients of this age group.

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