Assessment of the prevalence of osteoporosis among children with hemophilia and its relation to serum 25(oh) vitamin D and serum rankl

Hamza Rami Mohamed¹, Amin Radwa Ezzat¹, Aboelmakkarem Hoda Ahmed*²

¹Faculty of Medicine, Cairo University, Cairo City, Arab Republic of Egypt
²Department of Pediatrics, Zagazig General Hospital, Zagazig City, Arab Republic of Egypt

Article History:
Received on: 07 Nov 2020
Revised on: 11 Dec 2020
Accepted on: 17 Dec 2020
Keywords:
Hemophilia,
Osteoporosis,
RANKL,
Vitamin D,
DEXA scan

ABSTRACT
Osteoporosis is one of the comorbidities that complicating hemophilia. Recurrent hemarthrosis, chronic arthropathy and immobilization all are factors that make patients with hemophilia prone to this complication. Till recently the major emphasis of the osteoporosis diagnosis is the dual-energy X-ray absorptiometry (DEXA) scan, however, the definition of osteoporosis in pediatrics not only low bone mineral density measured by DEXA scan but also required the presence of clinically significant fracture history. Investigation target is evaluating the osteoporosis prevalence between cases with hemophilia, assessing the risk factors increasing its incidence including diet habits, the severity of hemophilia, duration between the first diagnosis of the disease and enrollment in the study and chronic hepatitis C infection. Also, considering the role of vitamin D deficiency, and another potential indirect mechanism mediated through Receptor activator of NF-Kappa-B, the Receptor activator of NF-Kappa-B ligand (RANK-RANKL) pathway has been suggested in the pathogenesis of bone disease and osteoclastic activity but remains controversial. Thirty-nine hemophilia pediatric patients were recruited from hematology clinic, Cairo university hospital, history taking, and examination were done focusing on the musculoskeletal system and dietetic data. Twenty normal age-matching children enrolled as a control group. DEXA scan results showed normal bone mineral density in 28 patients (71.8%) and osteoporosis in 11 patients (28.2%). The median Z score of patients was -1.40 (-2.2 – -0.6). There was a statistically significant decrease of bone mineral density in patients with hemophilia comparing with a control group with P-value <0.001, also we observed significantly higher serum RANKL in the group of patients with low bone mineral density ensuring the relation between RANKL and osteoclastic activity.

*Corresponding Author
Name: Aboelmakkarem Hoda Ahmed
Phone:
Email: ahmadhoda550@gmail.com

INTRODUCTION
Hemophilia is a coagulation disorder that causes acute hemorrhages in weight-bearing joints (Gurcay et al., 2006). It results from mutations in factor VIII gene led to a partial or complete reduction in correlated protein. VIII reduction (Hemophilia A) accounts for 85% of patients and 15% are for factor IX reduction (Hemophilia B) (Rodriguez-Merchan, 2003). Bleeding tendency causes complications varies from hemorrhrosis to intracranial hemorrhage (Kashyap and Choudhry, 2001). Acute bleed-
ing led to increasing synovial cavity pressure and led to many pain. Intraarticular bleeding causes a direct chemical effect on synovium, cartilage, and bone. Over time, blood hemosiderin becomes deposited in tissue. Recurrent hyperemia of joint in developing children led to juxta-articular osteoporosis and overgrowth (Barnes et al., 2004).

Severe hemophilia cases defined as clotting factor activities level of 1% or less, they tend to bleed more often and liable for the decrease in bone densities for many reasons as arthropathy, risk factors are prolonged immobilization and reduced physical activity in those cases lead to bones fragilities and fractures post minor trauma (Nair et al., 2007).

In developing countries, factor VIII replacement was on-requiring post bleeding; the strategy unlike prophylactic factor replacement which was popular in many developed countries increase the frequency of hemarthrosis which by time complicated with osteoporosis (Christoforidis et al., 2011).

Osteoporosis represents as an important reason for morbidity in hemophilic cases. Osteoporosis diagnosis in children and adolescents NOT based of densitometric criteria’s alone as an address by International Society for Clinical Densitometry (ISCD), but needs both clinical fracture histories and low bone mineral content or bone mineral density.

Significant clinical fracture histories are one or more the following

1. Long bone fracture of the lower extremities.
2. Vertebral compression fracture.
3. Two or more long bone fractures of the upper extremities

Low BMD is bone mineral contents or areal BMD Z-score which less than or equal 2.0, adjusted for age, gender, and body size, as appropriate (Frank et al., 2007).

The etiology of this bone disease is multifactorial with special considerations to calcium and vitamin D. Recently, New cytokines took place in last 1990s, RANKL, RANK, and osteoprotegrin (OPG)Receptor activator belong to TNF and receptors superfamily. As TNF superfamily molecule, RANKL forms homotrimeric transmembrane proteins with an extracellular domain at carboxy-terminus (Nakashima et al., 2012). RANK and RANKL are very crucial for the development of osteoclasts and implications in the pathogenesis of postmenopausal osteoporosis and metabolic bone disease (Kong et al., 1999). Little data are available about this marker among hemophilia children.

Investigation target is characterized by the possible roles of RANKL and vitamin D in haemophilia-related bone loss.

**Method**

This study is a case-control study. It was carried out on 39 patients with hemophilia recruited from the hematology clinic. This group was compared with 20 control subjects. Their data that were selected from comparable healthy control Egyptian children and adolescents.

Verbal consent was obtained from patients or their parents or legal guardians before enrollment in the study, and our study plan was approved through an ethical committee.

**Participants**

All participants included in our investigation subjected to

**Complete histories take stressing on the following**

1. Age
2. Residence
3. Hemophilia severity for the patients
4. Dietetic history (illustrated in detail later)
5. Age of the first diagnosis of the disease for the patients
6. Frequency of receiving treatment for the patients

**Dietetic history**

The dietary recommendations to prevent osteoporosis includes:

1. Regular safe sun exposure for adequate vitamin D
2. Moderate salt intake
3. Consume plenty of calcium-rich foods, such as milk, yogurt and cheese, every day, throughout life
4. Avoid soft drinks, coffee, and tea (Ross et al., 2011).

In our study, we based our dietetic scoring among participants according to compliance to the previous dietary recommendations, in which participants who have this diet are considered having a healthy diet, and those who are not regularly receiving this diet to have an unhealthy diet.

Data on the most recent calcium, phosphorus; history of boney pains and ultrasound of affected joint to assess presence and severity of effusion; serologic
Table 1: Demographic data and Anthropometric measures of the cohort groups

|                               | Patients group No. = 39 | Control group No. = 20 | Chi-square test \( X^2/\text{t}*/z* \) | P-value |
|-------------------------------|-------------------------|------------------------|--------------------------------------|---------|
| Age in years Mean ± SD        | 9.23 ± 4.36             | 9.40 ± 4.02            | -0.145*                              | 0.885   |
| Weight Median (IQR)           | 26 (19 – 45)            | 21 (18 – 26.5)         | 1.886*                               | 0.059   |
| Height Mean ± SD              | 130.18 ± 25.20          | 119.15 ± 15.35         | 1.790*                               | 0.079   |
| Weight (percentile) Median (IQR) | 50 (25 – 75)         | 50 (10 – 75)           | 1.058*                               | 0.290   |
| Height (percentile) Mean±SD   | 45.72 ± 26.15           | 49.25 ± 24.19          | 0.503*                               | 0.617   |
| BMI (Kg/m2) Mean±SD           | 11.79 ± 3.90            | 12.30 ± 4.13           | 0.462*                               | 0.646   |
| Residence Urban               | 1 (2.6%)                | 2 (10.0%)              | 1.515*                               | 0.218   |
|                               | Rural                   | 38 (97.4%)             |                                     |         |

*: Independent t-test*: Mann-Whitney test

Table 2: Descriptive statistics for patients' group

|                                | Total no. 39 |
|--------------------------------|--------------|
| Duration of disease Mean ± SD  | 6.36 ± 3.86  |
| Diet (Diary products) healthy diet | 22 (56.4%)   |
| unhealthy diet                 | 17 (43.6%)   |
| Type of hemophilia A           | 35 (89.7%)   |
|                                | 4 (10.3%)    |
| Severity of hemophilia Mild    | 8 (20.5%)    |
| Moderate                       | 12 (30.8%)   |
| Severe                         | 19 (48.7%)   |
| Frequency of ttt On-demand treatment | 29 (74.4%)  |
| Regular/wk                     | 10 (25.6%)   |
| Bone pains Yes                  | 35 (89.7%)   |
| No                             | 4 (10.3%)    |
| Joint U/S (affected joint) Lt ankle | 2 (28.6%)  |
| Lt knee                        | 2 (28.6%)    |
| Rt knee                        | 3 (42.9%)    |
| Severity of joint effusion Mild | 1 (14.3%)    |
| Moderate                       | 3 (42.9%)    |
| Severe                         | 3 (42.9%)    |
| HCV Negative                   | 34 (87.2%)   |
| Positive                       | 5 (12.8%)    |
| HBV Negative                   | 39 (100.0%)  |
| Positive                       | 0 (0.0%)     |
### Table 3: Comparison between patients’ group and control group regarding DEXA scan

| DEXA Scan                  | Patients n. | Control n. | Q X² | P     |
|----------------------------|-------------|------------|------|-------|
| Normal                     | 28 (71.8%)  | 20 (100.0%)| 6.934| 0.031 |
| Osteoporosis               | 11 (28.2%)  | 0 (0.0%)   |      |       |
| BMD Mean ± SD             | 0.61 ± 0.18 | 0.98 ± 0.10| 8.543| <0.0001|
| Z-score [Median (IQR)]    | -1.40 (-2.2 - -0.6) | 0.75 (0.20 - 1.25) | 5.471 | <0.0001 |

### Table 4: Comparison between abnormal mineral density and normal mineral density groups regarding the studied parameters

| Parameter                        | Normal mineral density n. = 28 | abnormal mineral density n. = 11 | Chi-square test |
|----------------------------------|---------------------------------|---------------------------------|-----------------|
| Diet                             | Healthy diet 15 (53.6%)          | 7 (63.6%)                       | X²/t*            |
|                                  | Unhealthy diet 13 (46.4%)        | 4 (36.4%)                       | 0.325 0.568     |
| Severity of hemophilia          | Mild 6 (21.4%)                   | 2 (18.2%)                       | 0.230 0.891     |
|                                  | Moderate 8 (28.6%)               | 4 (36.4%)                       |                |
|                                  | Severe 14 (50.0%)                | 5 (45.5%)                       |                |
| Height (percentile)             | Mean ± SD 48.15 ± 27.22          | 43.64 ± 21.69                   | 0.489 0.628     |
|                                  | Range 5 – 95                     | 5 – 75                          |                |
| Weight (percentile)             | Mean ± SD 45.74 ± 30.53          | 37.27 ± 31.81                   | 0.766 0.448     |
|                                  | Range 5 – 95                     | 5 – 90                          |                |
| BMI (Kg/m2)                     | Mean ± SD 11.89 ± 3.72           | 11.55 ± 4.50                    | 0.248 0.806     |
|                                  | Range 7 – 24                     | 2 – 17                          |                |
| Ca (mg/dl)                      | Mean ± SD 9.94 ± 0.61            | 10.00 ± 0.89                    | -0.245 0.808    |
|                                  | Range 8.9 – 11                   | 8.8 – 11.5                      |                |
| Po4 (mg/dl)                     | Mean ± SD 4.93 ± 0.68            | 4.58 ± 0.45                     | 1.532 0.134     |
|                                  | Range 3.4 – 6.5                  | 3.7 – 5.4                       |                |
| 25vit D (ng/ml)                 | Mean ± SD 13.96 ± 2.55           | 13.18 ± 1.89                    | 0.922 0.363     |
|                                  | Range 10 – 18                    | 10 – 16                         |                |
| RANKL (pg/ml)                   | Mean ± SD 128.86 ± 20.34         | 169.73 ± 6.44                   | -6.492 <0.001   |
|                                  | Range 93 – 160                   | 160 – 178                       |                |

### Table 5: Comparison between patients’ group and control group and normal serum 25 VIT D level

| 25 (OH) vit D | Patients group | Control group | Normal level | P1 | P2 | P3 |
|---------------|----------------|---------------|--------------|----|----|----|
| Mean ± SD     | 13.74±2.38     | 13.60±2.66    | 65.0±19.25   | T=11.829 | T=16.519 | T=0.211 |
| Range         | 10 – 18        | 10 – 18       | 30 – 100     | <0.001 | < 0.001 | 0.834 |
Table 6: Correlation between 25vit D and other studied parameters in the patient group (n = 39)

| Parameter         | R     | P     |
|-------------------|-------|-------|
| Age               | -0.099| 0.547 |
| Duration of disease | 0.028 | 0.865 |
| Height            | -0.017| 0.916 |
| Weight            | 0.032 | 0.848 |
| BMI (Kg/m2)       | 0.233 | 0.153 |
| RANKL (pg/ml)     | -0.112| 0.496 |
| Ca (mg/dl)        | 0.042 | 0.798 |
| Po4 (mg/dl)       | 0.228 | 0.163 |
| Z-score           | 0.204 | 0.213 |

Table 7: Comparison between patients’ group and control group regarding serum RANKL

| RANKL (pg/ml) | Patients group       | Control group      | Independent t-test | P     |
|--------------|----------------------|--------------------|-------------------|-------|
| Mean ± SD    | 140.38 ± 25.53       | 111.70 ± 20.36     | T=4.358           | <     |
| Range        | 93 – 178             | 85 – 156           |                   | 0.0001|

Table 8: Correlation between serum RANKL and other studied parameters in the patients’ group (n = 39)

| Parameter       | R     | P     |
|-----------------|-------|-------|
| Age             | 0.014 | 0.935 |
| Duration of disease | -0.103| 0.533 |
| Height          | 0.053 | 0.747 |
| Weight          | 0.029 | 0.863 |
| BMI (Kg/m2)     | -0.067| 0.686 |
| 25vit D (ng/ml) | 0.162 | 0.324 |
| Ca (mg/dl)      | -0.126| 0.443 |
| Po4 (mg/dl)     | -0.146| 0.376 |
| Z-score         | -0.498| <0.0001** |

status for hepatitis B; hepatitis C; and HIV were collected from patient files.

The quantitative determination of serum RANKL by a commercially available ELISA kit. The quantitative determination of serum 25 (OH) vitamin D hormone was done using the commercially available ELIZA kit, the 25OHD normal serum levels ≥30 ng/ml, insufficient (20 - 30 ng/mL), and deficient <20 ng/mL.

BMD assessments by DEXA scan (DPX-IQ) BMD of lumbar spine (L1- L4) measure. Absolute values converted to Z scores. BMD expressed in g/cm2 and bone mineral content in gram. DEXA result was categorized according to cut-off points for cases: Low BMD diagnosis defined as BMD Z score of 2 SD or more below mean value (less than -2) compared to age and sex-matched healthy controls. And normal BMD more than -2 (Frank et al., 2007).

Statistical Analysis

Using SSPS for analysis of our results and for comparing between 2 groups with qualitative data used Chi-square, Fisher exact used instead of Chi-square in any cell was found below 5. Comparing between 2 independent group regard to quantitative results with parametric distributions by Independent t-test and non-parametric distribution by using a Mann-Whitney test.

Spearman correlation coefficients for assessment the correlation in two quantitative parameters at the
RESULTS AND DISCUSSION

All patients were males and twenty age-matched normal males were included as a control group.

Descriptive statistics

Thirty-nine male pediatric hemophilia patients used in our research. Patients’ characteristics showed in Tables 1 and 2. Mean age is 9.23 ± 4.36 years. Thirty-nine patients with mild (8, 20.5%), moderate (12, 30.8%) and severe (19, 48.7%) hemophilia. Thirty-five and 4 cases diagnose with hemophilia A and B. 29 cases treated on-demand and rest receive prophylactic factor replacement therapy. Regarding diet rich in dairy products, 22 (56.4%) patients received a healthy diet while 17 (43.6%) patients received on the contrary unhealthy diet. During the study, only seven patients had intra-articular bleeding with active arthropathy, three of them with severe hemarthrosis that limiting their motion. Right knee is the most joint to be affected (42.9%). Chronic hepatitis C infection was present in five patients. There were no patients with positive HIV or HBV serology.

Age-matched normal children with a mean age (9.40 ± 4.02) were enrolled in this study as a comparative group. Demographic data and anthropometric measures of the control group were given in Table 1.

Prevalence of osteoporosis

BMD reported being normal in 28 cases (71.8%) while osteoporosis in 11 cases (28.2%). The median Z score of patients was -1.40 (-2.2 – -0.6). There was a statistically significant decrease of bone mineral density inpatient group than a control group with P-
value <0.001 (Table 3 and Figure 1).

BMD by clinical and laboratory findings showed in Table 4 and Figure 2. No significant correlation among hemophilia severities and BMD.

No significant relation between BMD and disease duration prior to study enrollment. A significant correlation between serum RANKL and BMD was found.

**Relation to serum 25 (OH) Vit D**

The data of both patients and control groups were analyzed. The mean of serum 25 (OH) Vitamin D levels was 13.74±2.38, 13.60±2.66 of patients and control group, respectively. There was a statistically significant decrease of serum 25VIT D of patients group and control group than normal serum level with p-value <0.0001, with no statistical difference regarding serum 25VIT D between patients’ group and control group.

Statistically positive correlation among 25 VIT D and both weight and height centiles (P= 0.916, 0.848). Age, duration of disease, BMI, serum RANKL, Z score had no statistically significant correlation (P = 0.547, 0.865, 0.153, 0.496, 0.213 respectively) (Tables 5 and 6 and Figure 3).

**Relation to serum RANKL**

We observed an association of statistical significance between patients’ group and control group regarding serum RANKL. Also, a significantly negative correlation among serum RANKL and Z-score was observed. While no statistically significant correlation found with any of other studied parameters. Thus, patients with low mineral density were found to have higher serum RANKL. (Tables 7 and 8 and Figures 4 and 5).

Hemophilia has a significant relation with secondary osteoporosis. It was associated with loss in bone mass according to the National Osteoporosis Foundation (NOF), USA (Scharrer and Schramm, 2005). Patients with hemophilia have reduced physical activity, chronic arthropathy, hepatitis C seropositivity, which predict lower peak bone mass (Wallny et al., 2007).

Investigation target was assessment the bone mineral density in hemophilia children use DEXA scan and relation to serum 25VitD and RANKL level.

Results of the current study show that there was a statistically significant decrease of bone mineral density inpatient group than a control group with P-value <0.001. Similar to our results, in cohort study included ninety adult hemophilia patients were done by (Katsarou et al., 2010), 86% of cases have pathologic BMD. 55.6% have osteoporosis and 30.5% have osteopenia. Also, (Gallacher et al., 1994) found a reduction in BMD in 19 hemophilia cases, and (Wallny et al., 2007) studied 62 hemophilia cases and found 70% have a reduction in BMD in the femoral neck by DEXA evaluation.

Also, the results of the current study show a statistically significant increase in serum RANKL in the case group compared to the control group. With noted a statistically significant increase of serum RANKL in low mineral density patients than normal mineral density patients among the case group (P-value <0.0001), which denotes increased serum RANKL in patients with increased osteoclastic activity.

Similar to our results, (Christoforidis et al., 2010) reported increase osteoclastic activities in hemophilia cases comparing with control and increase RANKL serum level. Also, (Daniela et al., 2012) studied 18 hemophilic cases and 16 osteoarthritis patients, decrease OPG expression in hemophilic arthropathy synovium comparing with OA. RANK and RANKL immune positivity’s cases are strongly inclining layers in hemophilic arthropathy synovial tissue. In agreement with our results, a study performed on 89 hemophilia A adult males (median age varies 44 years; 76). Sclerostin, RANKL was measured in patients and control serums. Patients with hemophilia had lower circulating sclerostin and higher levels of serum RANKL (Anagnostis et al., 2015). Interestingly, studies in rheumatoid arthritis patients have reported increase RANKL serum level, lower serum level of OPG (Fadda et al., 2015).

On the contrary, the study was done by (Katsarou et al., 2010) didn’t give any significant differences in serum RANKL level and OPG among hemophiliacs cases and control.

A somewhat surprising finding in our study that there is a statistically significant decrease of serum 25VIT D of patients group and control group than normal serum level with p-value <0.0001, with no statistical difference regarding serum 25VIT D between patients group and control group. All the participants in our study were Vitamin D deficient (<20 ng/mL).

However, our results would be justified by knowing that hypovitaminosis D is prevalent in Egypt which is attributed to low physical activity, obesity, false diet habits such as decrease on milk consumed, low maternal education level, and few periods of sun exposure. In an Egyptian study done by (Shady et al., 2016) included 200 children with a mean age between (10.39+.58) found a relatively high prevalence (11.5%) of vitamin D deficiency among the
apparently healthy young population.

Similar to our findings, in a study done by (Simpson and Valentino, 2013) included 86 hemophilic males, with (age 2-64 years) found that Vitamin D levels were reduced in 65% subjects, the authors advised Vitamin D and calcium supplementation as a simple and inexpensive intervention to address this complication and warranted further investigation.

It is evident in our study and the study done by (Gerstner et al., 2009) that low level in 25-hydroxyvitamin D in most cases. The median 25-hydroxyvitamin D levels were 28 ng/ml below 30/ ng ml (the essential serum level to prevent osteoporosis) (LeBoff, 1999). A study was done by (Linari et al., 2012) also, found 100% of their hemophilic patients had levels <30 ng/ml (median 20 ng ml).

Another finding was a positive correlation in 25 VIT D and both weight and height centiles of hemophiliacs.

To the best of our knowledge this the first study to correlate between height and weight centiles with a level of 25 (OH) Vit D in hemophilic patients and so further larger studies are required to establish our finding. However, different studies discussing the correlation between BMI and 25(OH) Vit D were conducted. One of these studies which followed middle-aged women into their late lives showed that lower 25VitD was associated with higher weight and BMI (Lehtinen et al., 2016).

Furthermore, studies in diabetic patients showed a negative correlation between 25 (OH) Vit D and body mass index (BMI) (Ahmet et al., 2012; Wortman et al., 2000) that highlighted a strong significant effect of obesity due to adipocyte sequestration of serum 25-(OH) VIT D.

CONCLUSIONS

In conclusion, it is important to diagnose osteoporosis early in patients with hemophilia as it may complicate future management. Early detection could be either by laboratory serum markers or DEXA scan. A lot of serum markers are newly emerging with promising results; however, till now, the only reliable method for diagnosis is DEXA scan. Prevention of osteoporosis is a crucial step in the proper management of hemophilia which obtained through encouraging good habits (such as a diet adequate in calcium and vitamin D and exercise) and discourages harmful ones such as immobilization. Rehabilitation and exercise play a vital role in helping to avoid risk factors for osteoporosis in young hemophilia patients. Osteoporosis ought to be promptly treated regardless of the underlying cause.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

Ahmet, C., Gul, G., Nazlig, K. 2012. Relation of obesity with serum 25 hydroxy vitamin D3 levels in type 2 diabetic patients. J Res Med Sci, 17(12):1119-1123.

Anagnostis, P, Vakalopoulou, S., Christoulas, D., et al. 2015. The Role of RANKL/Osteoprotegerin and Wnt Signaling Pathways in the Development of Osteoporosis in Patients with Hemophilia. Blood, 126(23):2284-2284.

Barnes, C., Wong, P., Egan, B., Speller, T., Cameron, F., Jones, G., Ekert, H., Monagle, P. 2004. Reduced Bone Density Among Children With Severe Hemophilia. Pediatrics, 114(2):e177–e181.

Christoforidis, A., Economou, M., Papadopoulou, E., et al. 2010. Bone Status of Children With Hemophilia A Assessed With Quantitative Ultrasound Sonography (QUS) and Dual Energy X-ray Absorptiometry (DXA). Journal of Pediatric Hematology or Oncology, 32(7):e259–e263.

Christoforidis, A., Economou, M., Papadopoulou, E., et al. 2011. Comparative study of dual-energy X-ray absorptiometry and quantitative ultrasonography with the use of biochemical markers of bone turnover in boys with haemophilia. Haemophilia, 17(1):e217–e222.

Daniela, M., Anna, F. M., Silvia, L. 2012. RANK-RANKL-OPG in Hemophilic Arthropathy: From Clinical and Imaging Diagnosis to Histopathology. J Rheumatol, 39(8):1678–1686.

Fadda, S., Hamdy, A., Abulkhair, E., Elsify, H. M., Mostafa, A. 2015. Serum levels of osteoprotegerin and RANKL in patients with rheumatoid arthritis and their relation to bone mineral density and disease activity. The Egyptian Rheumatologist, 37(1):1–6.

Frank, R., Horacio, P., Linda, D. M. 2007. Fracture Prediction and the Definition of Osteoporosis in Children and Adolescents: The ISCD Pediatric Official Positions. J Clin Densiton, 11(1):22–28.

Gallacher, S. J., Deighan, C., Wallace, A. M. 1994. Association of severe haemophilia A with osteoporosis: a densitometric and biochemical study. Q J Med,
Gerstner, G., Damiano, M. L., Tom, A., et al. 2009. Prevalence and risk factors associated with decreased bone mineral density in patients with haemophilia. Haemophilia, 15(2):559–565.

Gurcay, E., Eksioglu, E., Ezer, U., Tuncay, R., Cakci, A. 2006. Functional disability in children with hemophilic arthropathy. Rheumatology International, 26(11):1031–1035.

Kashyap, R., Choudhry, V. 2001. Management of hemophilia in developing countries. The Indian Journal of Pediatrics, 68(2):151–157.

Katsarou, O., Terpos, E., Chatzismalis, P., Provelengios, S., Adraktas, T., Hadjidakis, D., Kouramba, A., Karafoulidou, A. 2010. Increased bone resorption is implicated in the pathogenesis of bone loss in hemophiliacs: correlations with hemophilic arthropathy and HIV infection. Annals of Hematology, 89(1):67–74.

Kong, Y. Y., Yoshida, H., Sarosi, I. 1999. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature, 397(6717):315–323.

LeBoff, M. S. 1999. Occult Vitamin D Deficiency in Postmenopausal US Women With Acute Hip Fracture. JAMA, 281(16):1505–1511.

Lehtinen, S. J., Agelii, M. L., Hunsberger, M., Zetterberg, H., Lissner, L. 2016. Serum 25-hydroxy vitamin D levels in middle-aged women in relationship to adiposity and height trajectories over three decade. European Journal of Clinical Nutrition, 70(6):709–714.

Linari, S., Montorzi, G., Bartolozzi, D., et al. 2012. Hypovitaminosis D and osteopenia/osteoporosis in a haemophilia population: a study in HCV/HIV or HCV infected patients. Haemophilia, 19(1):126–133.

Nair, A. P., Jijina, F., Ghosh, K., Madkaikar, M., Shrikhande, M., Nema, M. 2007. Osteoporosis in young haemophiliacs from western India. American Journal of Hematology, 82(6):453–457.

Nakashima, T., Hayashi, M., Takayanagi, H. 2012. New insights into osteoclastogenic signaling mechanisms. Trends in Endocrinology and Metabolism, 23(11):582–590.

Rodriguez-Merchan, E. 2003. Orthopaedic assessment in haemophilia. Haemophilia, 9(1):65–74.

Ross, A. C., Manson, J. E., Abrams, S. A. 2011. Clarification of DRIs for calcium and vitamin D across age groups. J Am Diet Assoc, 111(10):1467.

Scharrer, I., Schramm, W. 2005. 36th Hemophilia symposium Hamburg. Orthopaedic treatment in hemophilic.

Shady, M. M. A., Youssef, M. M., El-Din, E. M. S. 2016. Predictors of Serum 25-Hydroxyvitamin D Concentrations among a Sample of Egyptian Schoolchildren. The Scientific World Journal, pages 1–7.

Simpson, M. L., Valentino, L. A. 2013. Vitamin D Deficiency and Osteoporosis In Hemophilia: An Underappreciated Risk. Blood, 122(21):3593–3593.

Wallny, T. A., Scholz, D. T., Oldenburg, J., et al. 2007. Osteoporosis in haemophilia an underestimated comorbidity. Haemophilia, 13(1):79–84.

Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., Holick, M. F. 2000. Decreased bioavailability of vitamin D in obesity. The American Journal of Clinical Nutrition, 72(3):690–693.