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

Hamza Rami Mohamed[1], Amin Radwa Ezzat[1], Aboelmakkarem Hoda Ahmed*[2]

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

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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

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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 hemarthrosis 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 homotrimerictrans membrane 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/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%)	18 (90.0%)		

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

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		Control		Q	
	n.	%	n.	%	X^2	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

		Normal mineral density	abnormal mineral density	Chi-square test	
		n. = 28	n. = 11	X^2/t*	P
Diet	Healthy diet	15 (53.6%)	7 (63.6%)	0.325	0.568
	Unhealthy diet	13 (46.4%)	4 (36.4%)		
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	13.1 8 ± 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)

	25vit D 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 t	P
Mean ± SD Range	140.38 ± 25.53 93 – 178	111.70 ± 20.36 85 – 156	T=4.358	< 0.0001

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

	RANKL 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) compare 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 inn expected count 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

same groups.

RESULTS AND DISCUSSION

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

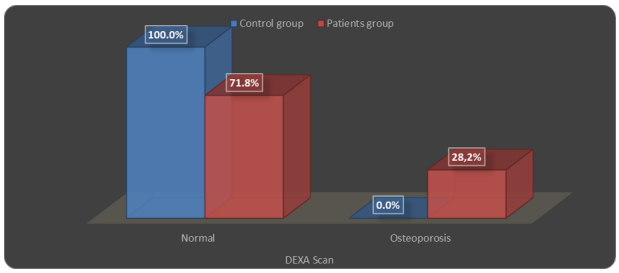

Figure 1: DEXA scan of the study groups.

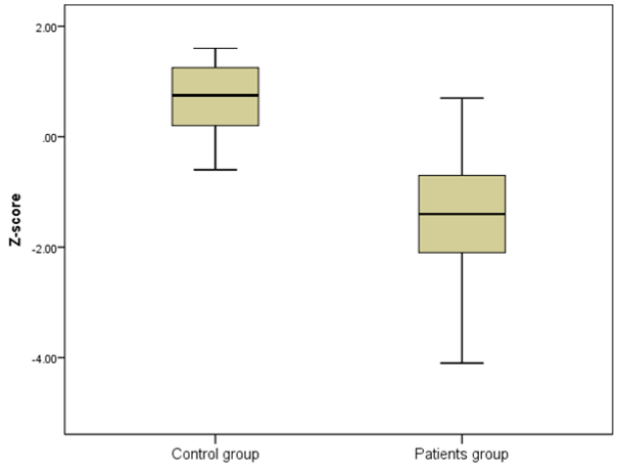

Figure 2: Z- score measures of the study groups.

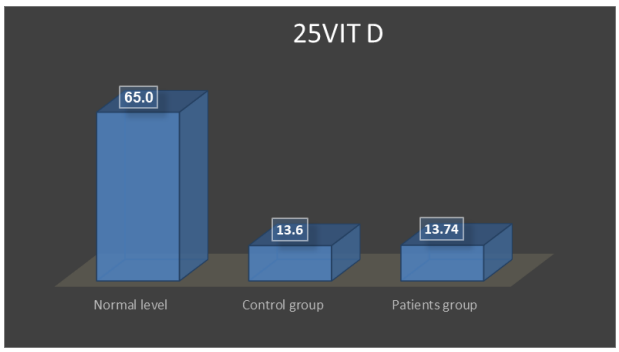

Figure 3: Serum 25 VIT D of the study groups and its normal serum level.

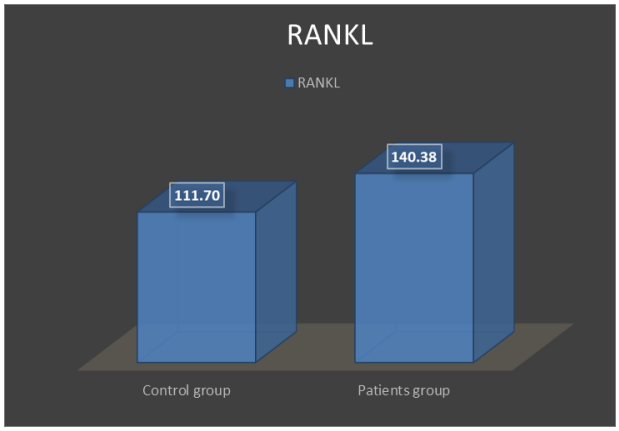

Figure 4: Serum RANKL of the study groups.

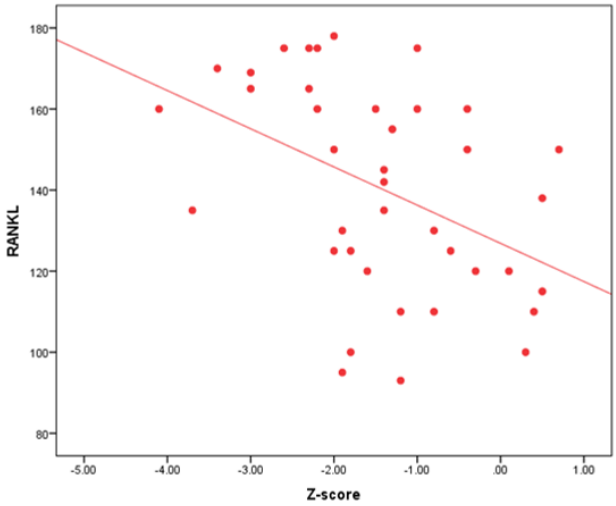

Figure 5: Correlation between serum RANKL and Z-score measures inpatient group.

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 4cases 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 28cases (71.8%) while osteoporosis in 11cases (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 25Vit D 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 had moderate hemophilia and underwent DEXA 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 inlining 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 hemophiliac 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; Wortsman *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.

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