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# Association between the levels of serum vitamin D and trace elements and joint health in children with hemophilia

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Hemophilia is defined as X-linked recessive bleeding disorder. Recurrent bleeding episodes lead to hemarthrosis. Objectives: to investigate the levels of serum 25(OH) D and trace elements in children with hemophilia A and B and to identify the possible association of these factors with Hemophilia Joint Health Score (HJHS). This case-control study was conducted among children with hemophilia A and B. A total of 48 cases were recruited from the hematology units at the Menoufia University Hospital ($n$ = 36) and Sohag University Hospital ($n$ = 12) from December 2020 to February 2022. Forty healthy controls were matched to cases on age, sex and socioeconomic status. Serum zinc and magnesium levels in the hemophilia patients were significantly lower than in the controls, while serum alkaline phosphatase levels in the cases were significantly higher than in the controls. Informed consent was obtained from all the children's parents and ethical approval was acquired from the ethical committee (ID: 5/2020PEDI38), Faculty of Medicine, Menoufia University. The levels of phosphorus and calcium were the same in two groups. Serum 25(OH) D levels were deficient in 85.4% of the cases and insufficient in 14.6%. None of the hemophilia patients had sufficient levels of serum 25(OH) D. There was no significant correlation between HJHS and the levels of serum trace elements but there was a significant positive correlation between HJHS and annualized bleeding rate and a significant negative correlation between HJHS and serum vitamin D. There was no significant difference regarding the demographic data except for weight and body mass index. The patients had significantly higher weight and body mass index compared to the control group. The levels of serum vitamin D and trace elements were decreased in hemophilia patients, and these low values were associated with the worst joint health.
**Key words:** *hemarthrosis, Hemophilia Joint Health Score, hemophilia, target joint, vitamin D*

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Hemophilia is an X-linked genetic coagulopathy with an estimated frequency of 1 in 10,000 people. The most prevalent types of hemophilia are factor VIII deficiency, or hemophilia A, which accounts for about 80% of all cases, and factor IX deficiency, or hemophilia B, which accounts for about 20% of cases [1]. Depending on residual endogenous levels of FVIII/FIX, patients with factor levels < 1 IU/dL are classified as having severe hemophilia and account for almost half of all cases; those with factor levels of 1–5 IU/dL and > 5 IU/dL have moderate and mild hemophilia, respectively. Even in patients with severe hemophilia, the bleeding phenotype may vary [2]. Clinical signs include abrupt bleeding throughout the body, especially in the joints and muscles. Other problems such as arthropathy are frequent complications of bleeding episodes [3]. The frequency of hemarthrosis in people with hemophilia ranges between 75 and 90%, with ankles and knees being the most affected joints [4]. The fear of bleeding reduces mobility and weight-bearing activity, which leads to decreased bone mineral density (BMD) [5]. Long-term immobility as well as hepatitis C virus and human immunodeficiency virus are predisposing factors that reduce BMD. Chronic inflammation associated with chronic hepatitis C virus and human immunodeficiency virus infection has deleterious effect on bone metabolism leading to the modulation of the bone-remodeling pathway through pro-inflammatory cytokines such as tumor necrosis factor-$\alpha$, interleukin (IL)-1, IL-6 and IL-17 that can promote the differentiation of osteoclast from their precursor cells. This results in imbalance between bone resorption and bone formation leading to osteoporosis and fractures [6]. Subclinical deficiencies of vitamin D, magnesium (Mg), and zinc (Zn) are also linked to reduced BMD and osteoporosis [7]. Few researches have discussed the significance of vitamin D in children with hemophilia, and their findings and conclusions considering vitamin D in children with hemophilia are unreliable. Even though some researchers observed reduced 25(OH) D levels in hemophilia cases compared to healthy controls, others found no difference [8]. The aim of our study was to investigate serum levels of 25(OH) D and some trace elements such as calcium (Ca), phosphorus (P), Zn and Mg in children with hemophilia A and B and to detect the possible association of these factors with Hemophilia Joint Health Score (HJHS).

## MATERIALS AND METHODS

This case-control study included 48 patients with hemophilia A and B who were recruited from the hematology units at the Menoufia University hospital (*n* = 36) and Sohag University hospital (*n* = 12) from December 2020 to February 2022 and 40 healthy controls who were matched to cases on age, sex, and socioeconomic status. Hemophilia cases were categorized according to the severity of their disease into mild (plasma factor levels > 5 IU/dL), moderate (1–5 IU/dL), and severe (< 1 IU/dL). All the subjects' medical histories were retrieved from the general questionnaire via in-person interviews. Informed consent was obtained from all the children's parents and ethical approval was acquired from the ethical committee (ID: 5/2020PEDI38), Faculty of Medicine, Menoufia University. To detect joint impairment, the HJHS version 2.1 was utilized. The HJHS comprises an assessment of the following features or items of the index joints (elbows, knees, and ankles): swelling, duration of swelling, crepitus on motion, muscle atrophy, extension and flexion loss, joint pain, strength, and global gait. The HJHS total score is calculated by adding the joint total scores to the global gait score. A total score of 0 represents the ideal joint health, while 124 indicates the worst joint health [9].

Blood samples (5 mL) were collected after 8–12 hours of overnight fast. After centrifugation, blood samples were kept at –80°C until laboratory analysis. To assess vitamin D status, serum 25(OH) D3 concentration was measured by enzyme-linked immunosorbent assay (ELISA); alkaline phosphatase (ALP), serum Ca, Mg, Zn, and P levels were measured using a colorimetric method. The quantitative determination of vitamin D (SunRed Biotechnology Company ELISA kits, Infinite F50 Tecan, Austria GmbH): a double-antibody sandwich ELISA was used to determine the concentration of human 25-dihydroxy vitamin D3 (25(OH) D3). Zn concentration was measured using a colorimetric method: Zn in the specimen is chelated by zincon (2-caboxy-2'-hydroxy-5-Sulfoformazyl-benzene) in the reagent at alkaline pH. The formation of the complex is measured at 610 nm wavelength. In an alkaline solution, Mg ions combine with the metallochromic dye calmagite to form a chromophore which absorbs at 520 nm. Ca is excluded from the reaction by forming a compound with ethylene glycol bis (β-aminoethyl ether) – N, N tetracetic acid (EGTA). Inorganic phosphorous (which is present in serum as phosphate) forms a phosphomolybdate complex with molybdic acid. This complex is reduced by stannous chloride to a blue tint that may be measured colorimetrically. Assay system employs formic acid as a protein solubilizer and glycerol as a stabilizer. At pH 8.5, Arsenazo III (2, 2- [1, 8-Dihydroxy-3, 6-disulpho-bis (azo)] di benzene arsenic acid) reacts with Ca ions to form a colored complex. The strength of the resulting color is proportional to the Ca concentration in the sample.

### Sample size estimation

Sample size was calculated by using the following formula: $N = (Z\alpha + Z\beta)/C^2 + 3$, at a power of 0.8 and a confidence interval of 95%. The sample size was estimated to be 45 subjects.

### Statistical analysis

The data were collected and analyzed using SPSS software (Statistical Package for the Social Science, version 20; IBM Armonk, New York). Numeric data were presented as mean ± SD and compared using the Student t-test. Non-numeric data were presented as frequency or percentage and compared using a Chi-square test ($\chi^2$). Correlation analysis (using Pearson's method) was applied for measurement the strength of association between two quantitative variables. The correlation coefficient (r) measures the strength and direction of the linear relationship between two variables. A $p$-value < 0.05 was considered significant.

## RESULTS

Demographic and anthropometric data, as well as the type and severity of the disease are provided in *table 1*. The mean level of vitamin D was significantly lower in the cases than in the controls. The majority of hemophilia patients (85.4%) had deficient levels of vitamin D and none of them had sufficient levels, whereas the majority of healthy controls (65%) had sufficient levels of vitamin D. The hemophilia patients had significantly lower serum levels of Mg, Zn and significantly higher serum ALP levels in comparison to the control group. Both groups showed no significant differences in the levels of total serum Ca, ionized Ca and P ($p$ > 0.05). The most common complications in the patients with hemophilia were hemarthrosis (97.9%) and bone pain (52.1%). Iliopsoas and gluteal muscle hematoma occurred in three patients. One patient had calf muscle hematoma. Intracranial hemorrhage occurred in one patient (*table 2*).

There were insignificant differences in the levels of serum vitamin D and other trace minerals between the patients with mild and moderate disease and those with severe disease ($p$ > 0.05) as presented in *table 3*. It was noticed that the majority of both groups had insufficient levels of vitamin D.

There was a significant positive correlation between the serum vitamin D and ionized Ca levels

**Table 1**
Demographic and anthropometric data of the cases and controls

| Demographic data | Patients ($n$ = 48) | Controls ($n$ = 40) | $p$-value |
|---|---|---|---|
| Age, years | 9.78 ± 3.66 | 8.11 ± 3.35 | 0.21 |
| Male sex, *n* (%) | 48 (100) | 40 (100) | – |
| Weight, kg | 31.39 ± 10.78<br>30 (24.25–35.75) | 24.00 ± 9.99<br>25.0 (14.0–31.5) | 0.001 |
| Weight percentile, *n* (%):<br>< 5th percentile<br>normal<br>> 95th percentile | <br>3 (6.3)<br>44 (91.7)<br>1 (2.1) | <br>1 (2.5)<br>38 (955)<br>1 (2.5) | 0.907 |
| Height, cm | 129.14 ± 20.98 | 122.03 ± 21.09 | 0.117 |
| Height percentile, *n* (%):<br>< 5th percentile<br>normal | <br>7 (14.6)<br>41 (85.4) | <br>3 (7.5)<br>37 (92.5) | 0.24 |
| BMI, kg/m$^2$ | 18.38 ± 2.54 | 16.51 ± 5.11 | 0.028 |
| Class of BMI, *n* (%):<br>underweight<br>normal<br>overweight | <br>21 (43.8)<br>26 (54.2)<br>1 (2.1) | <br>18 (45)<br>22 (55.0)<br>0 (0.0) | 0.449 |
| Type of hemophilia and disease severity (*n* = 48) | | | |
| Type of hemophilia, *n* (%):<br>hemophilia A<br>hemophilia B | 45 (93.8)<br>3 (6.2) | | |
| Disease severity according to factor level, *n* (%):<br>mild > 5%<br>moderate 1–5%<br>severe < 1% | 1 (2.1)<br>6 (12.5)<br>41 (85.4) | | |

*Note. The data are expressed as frequency (percentage), mean (SD), range. p-value < 0.05 was considered significant.*

**Table 2**
Levels of serum vitamin D and trace elements in the patients and controls

| Parameter | Patients ($n$ = 48) | Controls ($n$ = 40) | $p$-value |
|---|---|---|---|
| Vitamin D, ng/mL | 14.99 ± 5.58 | 36.76 ± 15.55 | < 0.001 |
| Level of vitamin D, *n* (%):<br>sufficient > 30 ng/mL<br>insufficient 20–30 ng/mL<br>deficient < 20 ng/mL | <br>0 (0.0)<br>7 (14.6)<br>41 (85.4) | <br>26 (65)<br>9 (22.5)<br>5 (12.5) | < 0.001 |
| Total Ca, mg/dL | 8.85 ± 0.60 | 8.89 ± 0.43 | 0.09 |
| Ionized Ca, mg/dL | 1.22 ± 0.22 | 1.23 ± 0.11 | 0.56 |
| ALP, U/L | 223.01 ± 54.24 | 175.11 ± 59.45 | < 0.001 |
| Serum Mg, mmol/L | 0.39 ± 0.25 | 0.61 ± 0.23 | < 0.001 |
| Serum Zn, mg/dL | 95.79 ± 29.08 | 170.11 ± 51.34 | < 0.001 |
| Serum P, mg/dL | 2.45 ± 2.14 | 2.89 ± 2.11 | 0.43 |
| Complications and HJHS in the hemophilia patients | | | |
| Hemarthrosis, *n* (%) | 47 (97.9) | | |
| Bone pain, *n* (%) | 25 (52.1) | | |
| Iliopsoas muscle hematoma, *n* (%) | 3 (6.3) | | |
| Gluteal muscle hematoma, *n* (%) | 3 (6.3) | | |
| Calf muscle hematoma, *n* (%) | 1 (2.1) | | |
| Intracranial hemorrhage, *n* (%) | 1 (2.1) | | |
| HJHS | 50.02 ± 21.04 | | |

*Note. The data are expressed as mean (SD). P-value < 0.05 was considered significant.*

(r = 0.33, $p$ = 0.03), a significant negative correlation between the serum vitamin D and ALP levels and between the total serum Ca concentration and annualized bleeding ratio (ABR). We found a significant negative correlation between HJHS score and serum vitamin D levels (r = –0.31, $p$ = 0.03) and a significant positive correlation between HJHS score and ABR (r = 0.73, $p$ = 0.001) (*table 4* and *figure*).

## DISCUSSION

In our study, there was no significant difference in age between the patients and controls (9.78 ± 3.66 y.o. vs 8.11 ± 3.35 y.o.), sex (all participants were males) and height (129.14 ± 20.98 cm vs 122.03 ± 21.09 cm), but there was difference in weight and body mass index (BMI). The patients had significantly higher weight and BMI than the controls. Twenty-six (54.2%) of the studied patients and 21 (55%) of the controls had normal BMI. Twenty-one (43.8%) patients and 18 (45%) controls were underweight. One subject in each group was considered overweight. The weight and height of

**Table 3**
Levels of vitamin D and trace elements in the hemophilia patients according to the disease severity

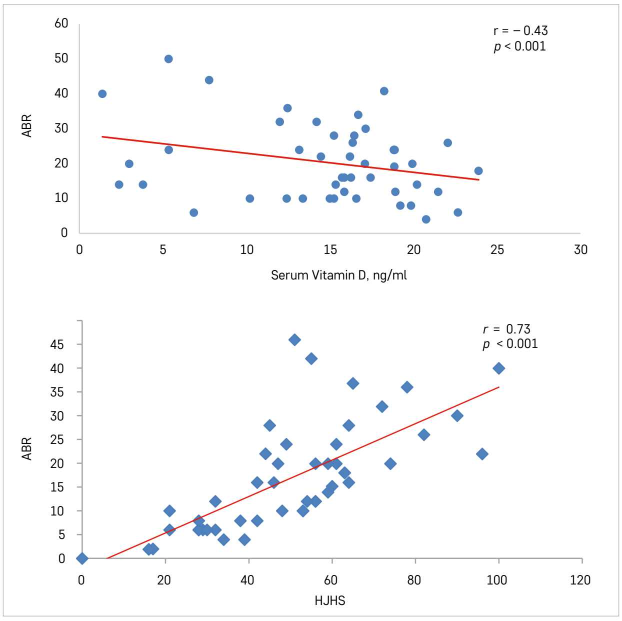
| Vitamin D and trace elements | Severity of hemophilia | | $p$-value |
|---|---|---|---|
| | Mild and moderate ($n$ = 7) | Severe ($n$ = 41) | |
| Vitamin D, ng/mL | 15.84 ± 6.54 | 14.85 ± 5.48 | 0.66 |
| Level of vitamin D, *n* (%):<br>insufficient 20–30 ng/mL<br>deficient < 20 ng/mL | <br>2 (28.6)<br>5 (71.4) | <br>5 (12.2)<br>36 (87.8) | |
| Total Ca, mg/dL | 8.61 ± 0.62 | 8.89 ± 0.60 | 0.25 |
| Ionized Ca,mg/dL | 1.21 ± 0.24 | 1.22 ± 0.21 | 0.98 |
| ALP, U/L | 221.66 ± 76.45 | 223.24 ± 50.78 | 0.94 |
| Serum Mg, mmol/L | 0.50 ± 0.45 | 0.37 ± 0.21 | 0.22 |
| Serum Zn, mg/dL | 93.28 ± 27.42 | 96.22 ± 29.67 | 0.80 |
| Serum P, mg/dL | 3.87 ± 2.32 | 2.21 ± 2.04 | 0.06 |

*Note. The data are expressed as mean (SD). p-value < 0.05 was considered significant*

**Table 4**
Correlation between the level of vitamin D and other parameters in the patients

| Parameter | r-value | p-value |
|---|---|---|
| Total Ca | –0.09 | 0.53 |
| Ionized Ca | 0.33 | 0.03 |
| ALP | –0.30 | 0.04 |
| Serum Mg | –0.12 | 0.38 |
| Serum Zn | 0.15 | 0.29 |
| Serum P | 0.13 | 0.22 |
| ABR | –0.43 | < 0.001 |
| Correlation between HJHS score and vitamin D and ABR in the studied patients | | |
| Vitamin D | –0.31 | 0.03 |
| ABR | 0.73 | < 0.001 |

**Figure**
Correlation between vitamin D and ABR (A), HJHS and ABR (B)




the majority of cases and controls were between the 5th and 95th percentile (normal). Abbasnezhad et al. [10] reported that there were insignificant differences in age, weight, BMI, and sociodemographic characteristics. Badr et al. [11] reported that among the studied hemophilia patients (30 males) age ranged from 6 to 22 years, the mean BMI was 20.98 ± 8.29, 50% of the studied cases were of healthy weight and 13.3% of them were obese.

As for the type of hemophilia, the majority (93.8%) of our cases had hemophilia A, and only three cases had hemophilia B. As for the severity of hemophilia, our study showed that only one (2.1%) patient had mild disease, 6 (12.5%) patients had moderate disease, and most (85.4%) of the patients had severe disease. Rodriguez-Santana et al. [12] reported that 79% of their patients had hemophilia A and 58% had severe hemophilia. The majority of the cases (82%) had no comorbidities and 77% of the patients had 1 to 5 bleeds per year. Payal et al. [1] reported that, out of a total of 56 cases, 51 (91.07%) cases were diagnosed with hemophilia A, and 5 cases (8.92 %) were diagnosed with hemophilia B. According to the factor level, 25 (44%) cases had severe disease, 20 (36%) had moderate disease, and 11 (20%) had mild disease. In the study by Varghese and Padmakumar [13], factor VIII deficiency was found in 30 (75%) cases (hemophilia A), while 10 (25%) cases had Factor IX deficiency (hemophilia B). The majority of these cases had severe deficiency.

In our study, the most common clinical presentations among hemophilia patients were bleeding after circumcision (35.4%), joint bleeding (97.7%) and easy bruising (20.8%). Two patients had epistaxis. Dental bleeding, hematuria, hematemesis, and wound bleeding occurred in one patient each. In our study, the mean annualized bleeding rate (ABR) was 20.56. Payal et al. [1] reported that, post-traumatic bleeding was the first clinical manifestation in 20 (36%) cases; the mean number of bleeds per year was 6.5 ± 9. Thirty cases (53.57 %) had 1–5 episodes of joint bleeding in the last year. Four (7.14 %) cases had more than 10 bleeding episodes. The median number of joint bleedings was 3 ± 5. Dorgalaleh et al. [14] found that, the most common clinical manifestations were hemarthrosis, epistaxis, ecchymosis, and post-dental extraction hemorrhage.

Oldenburg et al. [15] reported the model-based ABR and the median ABR for all bleeds in the episodic and prophylactic groups. Patel et al. [16] reported that after treatment the median number of joint bleeding events per six months reduced to 4 and the percentage of patients with target joints dropped to 42.1% (16 cases). The median number of target joints reduced from 6 to 1, and the median total joint range of motion score decreased from 16 to 5. Linari et al. [17] reported that the mean ABR was insignificantly higher in hemophilia A patients than in hemophilia B patients. Nevertheless, a significant correlation was discovered between ABR and the type and severity of hemophilia.

Young et al. [18] reported that in their study 46.4% of study subjects had no bleeding episodes.

There were no significant differences in CBC parameters between the cases and controls in the present study.

In our study, the mean level of serum vitamin D was significantly lower in the hemophilia patients than in the controls. The majority (85.4%) of the hemophilia patients had vitamin D deficiency and none of them had sufficient vitamin D levels. In contrast, the majority (65%) of the controls had sufficient vitamin D levels.

Albayrak and Albayrak [19] noted that blood vitamin D in children with hemophilia was considerably lower in winter and fall than in summer. The majority of hemophilia patients included in their study (96%) had insufficient vitamin D levels.

In the study by Alioglu et al. [20], the mean vitamin D levels were decreased in children with hemophilia when compared to controls. Ranta et al. [21] stated normal mean levels in hemophilia children with 50% insufficiency.

In the study by Eldash et al. [22], cases had considerably lower levels of Ca and vitamin D than healthy controls. Moderate and mild vitamin D deficiency was reported in 16 (43.2%) and 13 (35.1%) cases, respectively. Eight (21%) cases were shown to have normal vitamin D level.

Badr et al. [11] reported that the mean vitamin D level in the studied group was 18.13 ± 10.56 and 40% of hemophilia patients had severe deficiency of vitamin D. Abbasnezhad et al. [10] also reported that the number of subjects with vitamin D deficiency was higher in the hemophilia group than in the control group. In our study, hemophilia patients had significantly lower concentrations of serum Mg and Zn and significantly higher ALP in comparison to healthy controls. However, both groups showed no significant differences regarding the total levels of serum Ca, ionized serum Ca and serum P. Badr et al. [11] measured serum Ca, Ph and ALP among hemophilia patients: the mean of serum Ca was 9.04 ± 1.26, and the mean of serum ALP was 235.43 ± 73.54. In the study conducted by Abbasnezhad et al. [10], the serum levels of Zn, P, and Mg were significantly lower in patients with hemophilia than in healthy controls.

The results obtained by Ghaniema et al. [23] are in line with our findings: serum Zn and Mg were lower in hemophilia patients than in healthy subjects, with no significant difference in serum Ca values. The most common complications among the studied hemophilia patients were hemarthrosis (97.9%) and bone pain (52.1%). Iliopsoas and gluteal muscle hematoma occurred in three patients. One patient had calf muscle hematoma. Intracranial hemorrhage occurred in one patient. There was a significant negative correlation between HJHS score and serum vitamin D levels.

In the study by Borhany et al. [24], the most common type of bleeding was hemarthrosis which occurred in 72.85% of patients, followed by hematoma (51.4%), post-circumcision bleeding (37.14%), bleeding after trauma (28.51%). Less common bleeding episodes were associated with hematuria, bruising, and gum bleeding. In the Kumar et al. [25] study, 40.50% of patients had joint swelling at least once in their life, most commonly affecting the knee joint. Sajid et al. [26] also reported that the knee joint was the most frequently affected joint (48%) and that in 36% of cases more than one joint was affected. Abbasnezhad et al. [10] showed a strong negative correlation between circulating 25(OH) D levels and HJHS score.

In our study, there were no significant differences in the levels of serum vitamin D and other trace elements among the patients with mild, moderate and severe disease. The majority of hemophilia patients had insufficient levels of serum vitamin D. Badr et al. [11] demonstrated that there was a significant difference in the mean levels of vitamin D, vitamin D status and the severity of hemophilia. Similarly, Sanadhya and Singh [27] found a significant correlation between serum vitamin D levels and the severity of hemophilia. In their study, 97.14% of patients with severe hemophilia had insufficient vitamin D level, so they concluded that the severity of vitamin D deficiency increases with higher severity of hemophilia.

Linari et al. [17] obtained similar results in their research. Albayrak and Albayrak [19] found high levels of vitamin D deficiency in children with hemophilia which demonstrates that hemophilia cases are prone to vitamin D deficiency. Eldash et al. [22] proposed that elements associated with hemophilia may enhance the risk of vitamin D insufficiency in children with hemophilia. In their research there was a significant difference in vitamin D levels between hemophilia cases and controls and they suggested that this deficiency was caused by a combination of factors. Some of them may be attributable to a shorter exposure to sunlight, smaller area of exposure, and the depth of penetration. Reduced mobility and frequent hospitalizations due to arthropathy may also contribute to the deficit of sunlight. Rapid changes in Ca metabolism associated with patients' immobility and inactivity may also reduce vitamin D levels. We found that HJHS had no significant correlations with serum trace elements but it had a significant positive correlation with ABR. Abbasnezhad et al. [10] reported that serum concentrations of Zn, Mg and P had a negative association with HJHS, whereas serum Ca had no correlation with HJHS.

Ghaniema et al. [23] reported that in patients with hemophilia A blood levels of Zn were significantly negatively correlated with joint problems and the level of functional impairment. Serum levels of Mg showed no association with these factors, however. Alioglu et al. [20] found a strong negative correlation between serum 25(OH) D and total joint score in hemophilia patients. Young et al. [18] reported that in patients with one or more target joints at baseline, the median ABR for the 12 months before the study was 8.0.

As for the types of target joints, we found that there was no significant difference in the levels of vitamin D between patients with different target joints. In our study, 58.33% of the patients had only one target joint while 41.67% of patients had two or more target joints. Mishra et al. [28] found that the most common target joint was the knee (57.1% of cases). About 76% of patients had swollen joints resulting in restricted joint mobility. Bleeding in soft tissues and joints occurred 62.3% and 15.6% of patients, respectively. The percentage of severe hemophilia cases was higher in type A hemophilia patients.

## CONCLUSION

The majority of our patients had severe hemophilia A. Most of them had deficient levels of vitamin D, significantly low levels of serum Mg and Zn and significantly high levels of ALP. HJHS had no significant correlations with the levels of serum trace elements, but it had a significant negative correlation with serum vitamin D and a positive correlation with annualized bleeding rate.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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