Assessment of serum level of vitamin D in infants and children with Down syndrome

Manal M. El-Hawary[a], Shahira M El-Shafie[b], Heba El-Awady[a], Tamer Ragab[c], Raooth Nabile[a]

Departments of [a]Pediatric, [b]Clinical Pathology and [c]Cardiology, Faculty of Medicine, Fayoum University, Fayoum, Egypt

Correspondence to Heba El-Awady, MD, Department of Pediatrics, Faculty of Medicine, Fayoum University, Fayoum 012411, Egypt
Tel: +20 100 157 4770;
e-mail: hebawady@yahoo.com

Received 04 July 2018
Accepted 12 August 2018

Middle East Journal of Medical Genetics
2018, 7:104–111

Background
Vitamin D has multiple extraskeletal functions. Patients with Down syndrome (DS) are at more risk of vitamin D deficiency owing to multiple environmental and hormonal factors, so vitamin D supplementation plays a vital role in their lifestyle.

Objective
The aim of the study is to assess serum vitamin D level and to study the several factors that may affect its level in infants and children with DS.

Patients and methods
The study enrolled 50 children, where 30 of them were diagnosed as having DS (group I) and the other 20 were defined as a control group (group II). Detailed systemic examination was performed for all participants. Anthropometric measurements including weight, height, and head circumference were assessed. Blood samples were collected and evaluated for 25-hydroxy vitamin D level.

Results
The mean serum vitamin D level was 30.65 ± 20.64 in group I compared with 55.80 ± 22.79 in group II, with significant P value of less than 0.0001. In patients with DS, 6.7% were severely deficient (<10 ng/ml), 53.3% had insufficient serum vitamin D level (10–32 ng/ml), and 40% had adequate serum vitamin D level (>32 ng/ml). In group II, only 20% had insufficient serum vitamin D level and 80% had adequate level.

Conclusion
Vitamin D deficiency and insufficiency were more prevalent in patients with DS. Vitamin D insufficiency was also reported in the control group, which indicates that it is a common health problem even among healthy participants. Diet rich in vitamin D, adequate sun exposure, and vitamin D supplements prevent vitamin D deficiency.

Keywords:
Down syndrome, insufficiency, vitamin D deficiency

Middle East J Med Genet 7:104–111

2090-8571

Introduction

Down syndrome (DS) is the most common genetic disorder occurring in approximately one per 1000 babies born per year (Karaman, 2010). It is also known as trisomy 21, which is caused by the presence of a third copy of chromosome 21 (Weijerman and de Winter, 2010).

There are three types of DS, including nondisjunction 94% (classic or primary type), which is caused by failure of normal chromosome 21 segregation during meiosis; translocation 4%; and mosaic type 1% (Lana-Elola *et al.*, 2011).

Vitamin D is one of the fat-soluble vitamins that enhance the intestinal absorption of calcium, phosphate, and zinc (Hawli *et al.*, 2009). It is essential for bone health (Holick, 2006), calcium homeostasis, and skeletal mineralization (Ross *et al.*, 2013). Vitamin D also has immune-modulatory effects (Adams and Hewison, 2008).

Serum levels of 25-hydroxy vitamin D [25(OH)D] between 32 and 100 ng/ml are considered adequate, levels between 11 and 32 ng/ml are considered as vitamin D insufficiency, and levels less than 10 ng/ml are considered as severe deficiency (Mulligan *et al.*, 2010).

In DS, several hormonal and environmental factors may contribute to low bone mineral density, muscle hypotonia, low physical activity, low calcium and vitamin D intake, growth retardation, and thyroid dysfunction (Reza *et al.*, 2013; Ferry *et al.*, 2014).

Vitamin D plays a significant role in the health of such patients; thus, vitamin D deficiency predisposes them to bone fragility, osteoporosis, and fractures (Wahl *et al.*, 2012).

 DOI: 10.4103/MXE.MXE_10_18

Patients and methods

This is a descriptive case–control study designed to evaluate the serum level of vitamin D in infants and children with DS. The study enrolled 50 candidates aged 2–6 years. A total of 30 patients diagnosed as having DS were recruited from the Genetic outpatient clinic in Fayoum University Hospital in a period of 6 months from July 2015 to December 2015 (group I). The other 20 children were age-matched and sex-matched (group II).

DS cases taking vitamin D or calcium supplementation and patient having any other chronic illnesses that may affect vitamin D level such as skeletal diseases, renal diseases, hepatic diseases, severe obesity, severe illness, chronic malabsorption disorders, and neurological disorders were excluded.

Informed consent was obtained from patients' parents and guardians according to the guidelines of the Medical Research Ethics Committee of Fayoum University Hospital. Full history was taken, including detailed nutritional history (including type of feeding, time of weaning, and type of food introduced, stressing on food rich in vitamin D as egg yolk, liver, fish, fortified milk, and cereals), developmental history, and other important factors that may affect vitamin D synthesis such as exposure of the children to sunlight, physical activity, and number of hours they spent outdoors per week (0–2 h/week is considered as low physical activity and if more than 2 h/week is considered as adequate physical activity) (Wahl *et al.*, 2012).

Full physical examination including anthropometric measurement (weight, height, head circumference, and BMI), general examination and detailed systemic examination were performed, including respiratory, cardiac, abdominal, skeletal, and neurological systems.

Laboratory investigations including serum calcium, phosphorus, alkaline phosphatase (ALP), and 25(OH)D were assessed for all participants. Parathyroid hormone and imaging studies as echocardiography and pelviabdominal ultrasonography were performed for all DS cases.

Assessment of serum level of vitamin D

Vitamin D level is quantitated by solid phase enzyme-linked immunosorbent assay (ELISA) method, using ELISA kit from Sigma (3050 Spruce St, St. Louis, Missouri 63103, USA), based on the principle of competitive binding. In brief, 10 μl of 25(OH)D standards, controls, and samples was dispensed into each well, and then 200 μl of 1× working solution of biotinylated 25(OH)D reagent was added into each well. Contents were carefully mixed in the well for 20 s using a plate shaker at 200–400 rpm, and then the plate is incubated for 90 min at room temperature (10–26°C). After incubation, the plate is washed by 1× wash buffer for three times. Overall, 200 μl of enzyme conjugate (streptavidin-HRP) is then added into the well, and the plate is incubated for 30 min, at room temperature. After second intubation, plate is washed by 1× wash buffer for three times and 200 μl of TMB substrate is added into each well, and incubated for 30 min at room temperature, in the dark. Then, 50 μl of stop solution is dispensed into each well to stop the enzyme reaction. The absorbance of the resultant color is read on an ELISA reader at 450 nm. A dose–response curve was established from the data by plotting standard concentrations against corresponding absorbance. Values for unknown samples were obtained by interpolating the curve.

Statistical analysis of data

The collected data were organized, tabulated, and statistically analyzed using SPSS software statistical computer package version 18 (SPSS Inc., Chicago, Illinois, USA). For quantitative data, median and interquartile range were calculated. Mann–Whitney *U*-test was used to compare between the two groups of the study regarding different quantitative variables. For qualitative data, the number and percent distribution was calculated, and χ^2 or Fischer exact test, when appropriate, was used as a test of significance. For interpretation of the results of tests of which are significant, significance was adopted at P value less than 0.05.

Results

Group I (DS) included 18 males and 12 females. A total of 22 patients were from rural areas, whereas eight patients were from urban areas. Group II (control group) included nine males and 11 females. Seventeen participants were from rural areas, and the other three participants were from urban areas. The mean age of our patients in group I was 2.9 ± 1.6 years, whereas in group II, the mean age of the participants was 3.7 ± 1.1 years.

Anthropometric measurements among the two study groups are illustrated in Table 1. Height assessment in group I showed that 24 (80%) of 30 were below the third percentile and the other six (20%) were between 3rd and 95th percentile for age and sex. This showed significant difference when compared with control group ($P < 0.0001$). Head circumference measurements

Table 1 Anthropometric measurement among the study groups

Variables	<3rd percentile [*n* (%)]		Between 3rd and 95th percentile [*n* (%)]		*P*
	Group I	Group II	Group I	Group II	
Weight	2 (6.6)	0 (0)	28 (93)	20 (100)	0.0239
Height	24 (80)	0 (0)	6 (20)	20 (100)	<0.0001
Head circumference	15 (50)	0 (0)	15 (50)	20 (100)	<0.0001

showed that 15 (50%) of 30 patients in group I were below the third percentile and the other 15 (50%) were normal, with significant *P* value less than 0.0001.

BMI was calculated using the formula BMI = weight (kg)/height (m^2). DS age-related reference values for BMI were used (Cacciari *et al.*, 2006). Participants with a BMI between the 85th and 95th percentile were considered overweight. Participants with a BMI over the 95th percentile were considered obese (Broyles *et al.*, 2010). In group I, we found that two patients with DS were obese (above 95th percentile), and they had severe vitamin D deficiency. The other 28 DS patients had a normal BMI. In group II, one case was obese and had insufficient serum vitamin D level, two cases were overweight, and the other 17 cases had normal BMI.

In this study, we evaluated the factors that may affect serum vitamin D level such dietary contents of vitamin D, sun exposure, and physical activity among patients with DS and control participants (Table 2).

Detailed nutritional history was taken regarding breast/formula feeding, weaning, and type of food introduced, stressing on diet rich in vitamin D such as egg yolk, liver, fish, and meat. We found 76.7% of DS cases had a history of poor vitamin D dietary content when compared with control participants, with significant difference (*P* = 0.0001).

Regarding sun exposure, Holick *et al.* (2011) stated that the daily sun exposure should be for short periods uncovering forearms, hands, or lower legs and without sunscreen from late March or early April to the end of September, especially from 11 a.m. to 5 p.m. We found that most participants in both cases and controls had poor history of sun exposure, with insignificant *P* value.

Physical activity was assessed with a modified activity score composed of the scores for outdoor sports/leisure activities (Stagi *et al.*, 2013). We inquired about the number of hours nearly spent outdoors per week. If the child spent 0–2 h per week outdoors, low physical activity was considered, and if more than 2 h per week, adequate physical activity was considered. The results showed that most participants with DS had a history of low physical activity as compared with the control group, with significant *P* value of less than 0.0001.

Regarding vitamin D assessment, the mean level of vitamin D was 30.65 ± 20.64 ng/ml in patients

Table 2 Factors affecting serum vitamin D level among the study groups

	Group I (*N*=30) [*n* (%)]	Group II (*N*=20) [*n* (%)]	*P*
Vitamin D dietary content			
Diet rich in vitamin D	7 (23.3)	16 (80.0)	<0.0001
Diet poor in vitamin D	23 (76.7)	4 (20.0)	
Sun exposure			
Adequate sun exposure (11 a.m. to 5 p.m.)	5 (33.3)	7 (35.0)	0.903
Inadequate sun exposure	25 (66.7)	13 (65.0)	
Physical activity			
Low 0-2 h/week	24 (80.0)	3 (15.0)	<0.0001
Adequate>2 h/week	6 (20.0)	17 (85.0)	

with DS compared with 55.80 ± 22.79 g/ml in control group. This showed a statistically significant difference (*P*<0.0001) (Table 3 and Fig. 1).

In our study, two (6.7%) cases of DS showed severe deficiency below 10 ng/ml; one of them was a male and the other was a female. Both had cardiac anomalies. A total of 16 (53.3%) patients had insufficient serum vitamin D (11–32 ng/ml), comprising 10 females and six males. Twelve (75%) of them showed cardiac anomalies. The other 12 (40%) cases of DS had adequate level of serum vitamin D, ranging from 32 to 100 ng/ml. In comparison, only four children of the control group showed insufficient level between 11 and 32 ng/ml.

Echocardiographic findings in group I showed that 18 (60%) of 30 cases had congenital heart disease (CHD) in the form of Atrial septal defect (ASD), Ventricular septal defect (VSD), or both. ASD was the commonest CHD (66.6%). Only one control case had congenital VSD (Table 4).

Serum calcium, phosphorus, ALP, and parathyroid hormone assay were done for all participants. Parathyroid hormone assay was performed by ELISA. The reference level of Parathyroid hormone (PTH) ranges between 10 and 69 pg/ml. Four (13.3%) patients of group I had a high level of PTH and the others had normal levels. This showed no significant difference. Normal serum calcium level (9–11 mg/dl) was found in group I and group II. The mean serum calcium level in patients with DS was 9.69 ± 0.69 compared with 10.25 ± 0.85 in control group. This had a significant difference (*P* = 0.015). No significant differences were found in the mean serum phosphorus and ALP levels between the two groups; both groups had normal serum levels (Table 5).

Correlation was done between vitamin D level in DS cases and age, weight, height, and head circumference. Results showed a positive correlation between serum vitamin D level and head circumference among cases of DS (P = 0.030; Table 6 and Fig. 2).

Linear regression analysis was performed to show the different predictors affecting the serum vitamin D level in cases of DS. Head circumference and sun exposure were found to be significant predictors (P = 0.016 and <0.0001, respectively; Table 7).

Table 3 Vitamin D measurements results among the study groups

Variables	Group I (N=30) [n (%)]	Group II (N=20) [n (%)]	P
Vitamin D level (mean±SD)	30.65±20.64	55.80±22.79	<0.0001
Severe deficiency (<10 ng/ml)	2 (6.7)	0	0.017
Inadequate (11-32 ng/ml)	16 (53.3)	4 (20.0)	
Adequate (32-100 ng/ml)	12 (40.0)	16 (80.0)	

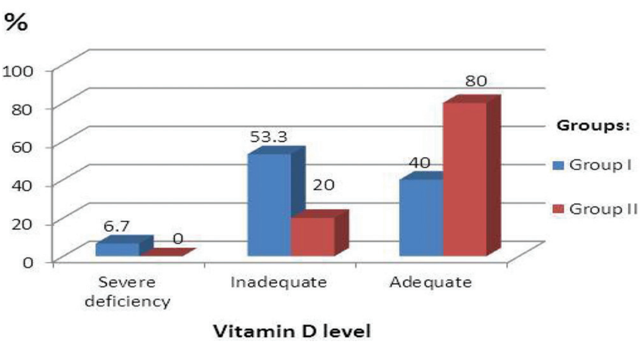
Table 4 The distribution of cardiac anomalies among Down syndrome cases (group II)

Variables	N (%)
ASD	12 (66.6)
VSD	3 (16.7)
Complex (ASD and VSD)	3 (16.7)

Table 5 Parathyroid hormone, serum calcium, phosphorus, and alkaline phosphatase levels among the study groups

Variables	Group I (N=30) (mean±SD)	Group II (N=20) (mean±SD)	P
PTH level (pg/ml)	26.55±42.67	28.86±21.27	0.058
Serum calcium level (mg/dl)	9.69±0.69	10.25±0.85	**0.015**
Serum phosphorus level (mg/dl)	3.57±0.50	3.55±0.51	0.910
Serum alkaline phosphatase	66.53 ± 9.87	65.10 ± 5.86	0.562

Figure 1



Serum level of vitamin D among the study groups.

No correlation was found between mean serum vitamin D level and other laboratory parameters among DS cases (Table 8).

Discussion

DS is a known cause of short stature, microcephaly, and growth retardation owing to chromosomal and endocrinal abnormalities. In our study, height and head circumference measurements in the two groups showed significant statistical differences, with significant P values of less than 0.0001. The same statistical difference was found in another study conducted by El Hayek *et al.* (2011).

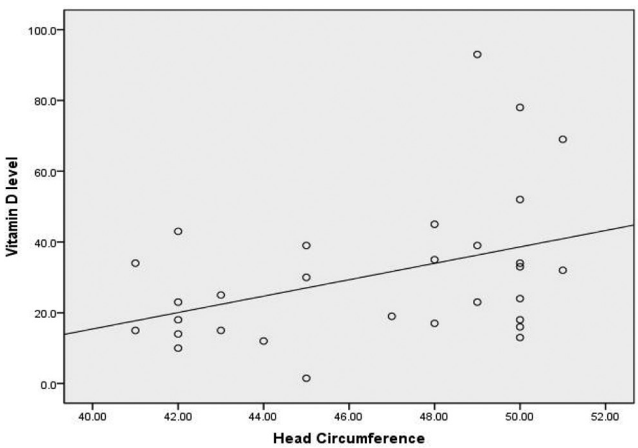
Annerén *et al.* (1990) and Karlberg *et al.* (1976) assumed that the growth pattern (weight, height, and head circumference) in patients with DS is characterized by an impaired growth velocity from birth until adolescence, especially during the age interval of 6 months to 3 years and during puberty. Regarding body weight, a nonsignificant P value was detected between the two groups (P = 0.239).

Regarding the BMI, only two DS cases were obese (BMI >95th percentile), and both had severe vitamin D deficiency. The relation between low serum vitamin D level and obesity was confirmed by Wortsman *et al.* (2000) who reported that obese patients have lower basal 25(OH)D levels.

Moreover, Alemzadeh *et al.* (2008) reported 55 cases with hypovitaminosis D in a group of 127 obese children aged 13 years.

Fiscella and Franks (2010) found that obese children and adolescents with DS were at a higher risk of a more

Figure 2



Correlation between the mean serum level of vitamin D and head circumference.

Table 6 Correlation between serum vitamin D level and study variables among Down syndrome cases

Study variable	Vitamin D level	
	r	*P*
Age	−0.275	0.141
Weight	0.317	0.088
Height	0.268	0.152
HC	0.398	0.030

HC, Head circumference

Table 7 Linear regression analysis for predictors of serum vitamin D level

	B	*t*	*P*
Constant	−50.648	−1.317	0.199
Age	−0.110	−0.750	0.911
Sex	0.008	0.053	0.958
Weight	0.173	0.811	0.424
Length	0.068	0.369	0.715
HC	2.095	2.576	0.016
Sun exposure	24.378	4.060	<0.0001
Vitamin D diet	0.175	1.131	0.268

Table 8 Correlation between vitamin D level and laboratory parameters among Down syndrome cases

	Vitamin D level	
	r	*P*
Parathyroid hormones	−0.195	0.301
Calcium	0.072	0.705
Phosphorus	−0.013	0.944
Alkaline phosphatase	−0.139	0.462

severe vitamin D deficiency. This deficiency is caused by decreased vitamin D bioavailability from cutaneous and dietary sources because of its deposition in body fat and because obese children may lead a more sedentary, indoor lifestyle. The study of Abu Shady *et al.* (2016) reported that obesity is an important predictor of low serum vitamin D level in prepubescent schoolchildren in Egypt.

We found no significant relation between different levels of serum 25(OH)D and age and sex ($P > 0.689$ and <0.819, respectively). On the contrary, Houghton *et al.* (2014) reported vitamin D insufficiency in females more than males. Moreover, Abu Shady *et al.* (2016) found that boys had higher 25(OH)D levels than girls, who had a significantly increased risk of vitamin D deficiency.

Regarding cardiovascular system, vitamin D deficiency is prevalent in neonates with congenital cardiac defects. Furthermore, lower postoperative 25(OH)D levels are associated with the need for increased inotropic support in neonates undergoing cardiac operations. These findings support that vitamin D deficiency plays an important role in myocardial injury and postoperative recovery (Graham *et al.*, 2013). In our study, 18 (60.6%) of 30 patients with DS had cardiac anomalies. The most common CHD was ASD [12 (66.6%) patients], whereas three (16.6%) patients had VSD and three (16.6%) other patients had both ASD and VSD. Two patients with CHD had severe vitamin D deficiency and 12 had inadequate serum vitamin D level. In comparison, a study performed by Freeman *et al.* (2008) and Vis *et al.* (2009) showed that the most common CHD in DS was the atrioventricular septal defect, with a prevalence of 45%, followed by ventricular septal defect in 35% and an isolated atrial septal defect in 8% of the cases. To our knowledge, there was no previous dedicated study of vitamin D assessment in DS cases with CHDs.

Regarding breast and formula feeding especially during the first year of life, we found no significant difference between the two groups ($P = 0.349$). Both breast and formula milk are deficient in vitamin D. This ensures the importance of vitamin D supplementation starting from the first day, and also the importance of vitamin D-rich sources in our dietary intake. These findings were similar to the study conducted by Heaney *et al.* (2003).

The study conducted by Thatcher *et al.* (2013) showed that most mothers with DS did not introduce vitamin D-rich sources in their diet as they were not oriented toward the importance of this information. We observed a similar situation in our study, as 76.7% of DS cases had a diet poor in vitamin D. In addition, the low economic state played an important role in this issue.

Vitamin D synthesis is strongly related to adequate sun exposure from 11 a.m. to 5 p.m., and so inadequate sun exposure represents a major risk of vitamin D deficiency (Holick, 2011). Surprisingly, although Egypt is a sunny country all the year, we found that most candidates in both groups showed history of inadequate sun exposure ($P = 0.903$). We found a strong relation between inadequate sun exposure and low serum vitamin D level, with significant *P* value less than 0.0001.

Lower serum levels of vitamin D were reported in different countries in healthy individuals such as 39% in Europe, 49.4% in Italy, 51.4% in Korea, and 56.4% in USA. This difference may be because of the lack of sunny climate in Europe and USA (González-Gross *et al.*, 2012).

The degree of physical activity was assessed based on asking about the number of hours the participants spent in sports. If they spent 0–2 h per week, low physical activity was considered, whereas more than 2 h per week, an adequate physical activity was considered. Low activity was the dominating feature in patients with DS when compared with normal healthy ones with significant ($P < 0.0001$).

Our data confirmed that patients with DS commonly spent less time outdoors and less time being physically active, but there was no relation ($P = 0.284$) between these findings and serum vitamin D level, which is in contrast to the results of Gordon *et al.* (2004) and Valtueña *et al.* (2013), who reported that adequate serum vitamin D level is strongly related to adequate physical activity. Bass *et al.* (2007) reported the relation between adequate physical activity and improved bone mass in patients with DS. Abu Shady *et al.* (2016) confirmed the relation between an adequate physical activity and sufficient serum vitamin D level.

In our study, assessment of serum level of 25(OH)D in group I revealed that two (6.6%) cases were severely deficient (<10 ng/ml), 16 (53%) cases had insufficient level (10–32 ng/ml), and the remaining 12 (40%) cases had adequate level of vitamin D, which is more than 32 ng/ml. More evident vitamin D deficiency in DS was published by Stagi *et al.* (2013) who reported that of 31 patients with DS, 10 (32.2%) had severe deficient serum 25(OH)D level, 19 (61.2%) had insufficient level, and only two (6.5%) had adequate level.

In group II, four (20%) children had an insufficient serum vitamin D level, whereas the other 16 (80%) participants had adequate level ($P = 0.017$). A recent study performed by Abu Shady *et al.* (2016) in Egypt showed that between 200 prepubescent schoolchildren, vitamin D deficiency was detected in 11.5% whereas insufficiency was detected in 15% of them.

Serum calcium level in DS cases was found to be significantly lower than in the control group ($P = 0.015$), although within the reference range. Moreover, serum PTH, phosphorus, and ALP levels were in the reference range.

No correlation was found between the mean serum vitamin D level and other laboratory parameters (serum calcium, phosphorus, ALP, and PTH). This agrees with the results reported by Stagi *et al.* (2013). However, Abu Shady *et al.* (2016) found that serum 25(OH)D levels were inversely related to PTH and serum phosphorus. Regarding serum calcium level, our results agree with Abu Shady *et al.* (2016) who found that low serum calcium level was not identified as a risk factor for vitamin D deficiency or insufficiency. Zubillaga *et al.* (2006) showed consistent relation between DS and vitamin D deficiency that can be corrected by vitamin D supplementation, whereas calcium and phosphorus levels were in the reference range.

We found no significant relation between different levels of serum 25(OH)D and age and sex ($P < 0.689$ and <0.819, respectively). However, Houghton *et al.* (2014) reported vitamin D insufficiency in females more than males. Moreover, Abu Shady *et al.* (2016) found that boys had a higher 25(OH)D levels than girls, who had a significantly increased risk of vitamin D deficiency.

In our study, serum vitamin D level positively correlated with head circumference measurements ($r = 0.398$, $P = 0.030$). Moreover, we found that head circumference measurements and adequate sun exposure were predictors of serum vitamin D level.

Low physical activity, inadequate sun exposure, and lack of vitamin D-rich diet represent important predictors of serum vitamin D level. The study performed by Abu Shady *et al.* (2016) in Egypt confirmed the relation between these factors and serum vitamin D level. These findings were also confirmed by Bener *et al.* (2009), Arguelles *et al.* (2009), and Saki Dabbaghmanesh *et al.* (2015).

Regarding the cytogenetic types of DS, 26 (86.6%) patients were of nondisjunction type, one (3.3%) was of translocation type, and three (10%) patients were of mosaic DS type. Moreover, another study performed in Brazil about the cytogenetic profile of DS cases showed that among 387 cases of DS, 92.2% had free trisomy, 6.2% had translocation involving chromosome 21, and 1.5% were mosaics (Devlin and Morrison, 2004). This is consistent with a study performed in Algeria which showed that among 22 cases with DS, free trisomy was presented in 20 (91%) cases, one (4.5%) case had translocation, and another one had mosaic DS (Qahatani *et al.*, 2011).

Regarding maternal age, 16 (53%) cases were between 35 and 40 years old in patients with DS whereas in the control group, only six (30%) participants were in the same age group, with insignificant difference ($P = 0.266$). Our results correlate this genetic disorder with the maternal age as one major risk factor, which is similar to the study of Parker *et al.* (2010). However, in the past ten years, some researchers documented a rise in the percentage of DS babies born by young mothers (<35 years of age). In China, Zheng *et al.* (2009) reported a high rate of DS births among young mothers.

Conclusion

This study showed high prevalence of insufficient serum vitamin D level in infants and children with DS compared with the control group. Insufficient serum vitamin D level was closely associated with many risk factors including, inadequate sun exposure, lack of physical activity, decreased introduction of vitamin D rich diet, and inadequate vitamin D supplementation. However, insufficient serum vitamin D level was also found in control healthy children.

Recommendations

More attention should be paid in Egypt to raise the awareness of vitamin D and its importance for bones and other body systems.

Health education about the value of proper sun exposure, physical activity, and healthy diet especially rich in vitamin D, and mother health education about the importance to avoid obesity, as it strongly affects serum vitamin D level, should be disseminated.

Special care and attention should be directed toward infants and children of DS. They should be informed about the importance of adequate serum vitamin D level in improving their lifestyle and the hazards of its deficiency.

Vitamin D supplementation is recommended to all infants and pregnant females as early as possible to guard against vitamin D deficiency.

Acknowledgements

The authors would like to thank all participants and their families for their active role in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

Abu Shady M, Youssef M, Shehata M, Salah El-Din E, El Malt H (2016). Association of serum 25-hydroxyvitamin D with life style and dietary factors in Egyptian prepubescent children. *Open Access J Med Sci* **3**:80–84.

Adams J, Hewison M (2008). Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab* **4**:80–90.

Alemzadeh R, Kichler J, Babar G, Calhoun M (2008). Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metabolism* **57**:183–191.

Annerén G, Gustavson K, Sara V, Tuvemo T (1990). Growth retardation in Down syndrome in relation to insulin-like growth factors and growth hormone. *Am J Med Genet Suppl* **7**:59–62.

Arguelles LM, Langman CB, Ariza AJ, Ali FN, Dilley K, Price H, *et al.* (2009). Heritability and environmental factors affecting vitamin D status in rural Chinese adolescent twins. *J Clin Endocrinol Metab* **94**:3273–3281.

Bass S, Naughton G, Saxon L, Iuliano-Burns S, Daly R, Briganti E, *et al.* (2007). Exercise and calcium combined results in a greater osteogenic effect than either factor alone: a blinded randomized placebo-controlled trial in boys. *J Bone Miner Res* **22**:458–464.

Bener A, Al-Ali M, Hoffmann G (2009). Vitamin D deficiency in healthy children in a sunny country: associated factors. *Int J Food Sci Nutr* **60**:60–70.

El Hayek J, Egeland G, Weiler H (2011). Vitamin D status of Inuit preschoolers reflects season and vitamin D intake. *J Nutr* **140**:1839–1845.

Ferry B, Gavris M, Tifrea C, Serbanoiu S, Pop AM (2014). The bone tissue of children and adolescents with Down syndrome is sensitive to mechanical stress in certain skeletal locations: a 1-year physical training program study. *Res Dev Disabil* **35**:2077–2084.

Fiscella K, Franks P (2010). Vitamin D, race, and cardiovascular mortality: findings from a national US sample. *Ann Fam Med* **8**:11–18.

Freeman S, Allen E, Oxford-Wright C, Tinker S, Druschel C, Hobbs C (2008). The National Down Syndrome Project: design and implementation. *Public Health Rep* **122**:62–72.

González-Gross M, Valtueña J, Breidenassel C, Moreno LA, Ferrari M, Kersting M, *et al.* (2012). Vitamin D status among adolescents in Europe: the healthy lifestyle in Europe by nutrition in adolescence study. *Br J Nutr* **107**:755–764.

Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ (2004). Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med* **158**:531–537.

Graham EM, Taylor SN, Zyblewski SC, Wolf B, Bradley SM, Hollis BW, *et al.* (2013). Vitamin D status in neonates undergoing cardiac operations: relationship to cardiopulmonary bypass and association with outcomes. *J Pediatr* **162**:823–826.

Hawli Y, Nasrallah M, Fuleihan G (2009). Endocrine and musculoskeletal abnormalities in patients with Down syndrome. *Nat Rev Endocrinol* **5**:327–334.

Heaney R, Dowell M, Hale C, Bendich A (2003). Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr* **22**:142–146.

Holick M (2011). Vitamin D deficiency in 2010, health benefits of vitamin D and sunlight: a D-bate. *Nat Rev Endocrinal* **7**:73–75.

Holick M, Binkley N, Bischoff-Ferrari H, Gordon CM, Hanley DA, Heaney RP, *et al.* (2011). Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **96**:1911–1930.

Houghton GA, Gray AR, Harper MJ, Winichagoon P, Pongcharoen T, Gowachirapant S, Gibson RS. (2014). Vitamin D status among Thai school children and the association with 1, 25-dihydroxyvitamin D and parathyroid hormone levels. *PLoS One* **9**:e104825.

Karaman A (2010). Medical problems in children with Down syndrome in the Erzurum area of Turkey. *Genet Couns* **21**:385–395.

Karlberg P, Taranger J, Engström I, Karlberg J, Landström T, Lichtenstein H, *et al.* (1976). I. physical growth from birth to 16 years and longitudinal outcome of the study during the same age period. *Acta Paediatr Scand Suppl* **258**:7–76.

Lana-Elola E, Watson-Scales SD, Fisher EM, Tybulewicz V. (2011). Down syndrome, searching for the genetic culprits. *Dis Model Mech* **4**:586–595.

Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. (2010). Implications of vitamin D deficiency in pregnancy and lactation. *Am J Obstet Gynecol* **202**:1–9.

Parker S, Mai C, Canfield M, Rickard R, Wang Y, Meyer R, *et al.* (2010). Updated National Birth Prevalence estimates for selected birth defects in the United States, 2004–2006. *Birth Defects Res A Clin Mol Teratol* **88**:1008–1016.

Qahatani M, Faridi W, Iqbal L, Nazia N, Riaz A (2011). Environmental, maternal and cytogenetic analysis of live born infants with Down's syndrome. *J US China Med Sci* **8**:143–149.

Ross A, Taylor C, Yaktine A, Del Valle HB (2013). *Dietary reference intakes for calcium and vitamin D.* Washington, DC: National Academies.

Reza SM, Rasool H, Mansour S, Abdollah H (2013). Effects of calcium and training on the development of bone density in children with Down syndrome. *Res Dev Disabil* **34**:3404–3409.

Saki Dabbaghmanesh M, Omrani G, Bakhshayeshkaram M (2017): *Vitamin D deficiency and its associated risk factors in children and adolescents in southern Iran.* Public Health Nutrition. 20:1851-1856.

Stagi S, Cavalli L, Iurato C, Seminara S, Brandi M, Martino M (2013). Bone metabolism in children and adolescents: main characteristics of the determinants of peak bone mass. *Clin Cases Miner Bone Metab* **10**:172–179.

Thatcher T, Fischer P, Tebben P, Singh RJ, Cha SS, Maxson JA, Yawn BP.

(2013). Increasing incidence of nutritional rickets. A population-based study in Olmsted County Minnesota. *Mayo Clinic Proc* **88**:176–183.

Valtueña M, González-Gross I, Huybrechts I, Breidenassel C, Ferrari M, Mouratidou T, *et al*. (2013). Factors associated with vitamin D deficiency in European adolescents. *J Nutr Sci Vitaminol (Tokyo)* **59**:161–171.

Vis J, Duffels M, Winter M (2009). Down syndrome: a cardiovascular perspective. *J Intellect Disabil Res* **53**:419–425.

Wahl DA, Cooper C, Ebeling PR, Eggersdorfer M, Hilger J, Hoffmann K, *et al*. (2012). A global representation of vitamin D status in healthy populations. *Arch Osteoporos* **7**:155–172.

Weijerman M, de Winter J (2010). Clinical practice. The care of children with Down syndrome. *Eur J Pediatr* **169**:1445–1452.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF (2000). Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* **72**:690–693.

Zheng C, Qin J, Du J, Chen K, Chen C, Tian X, *et al*. (2009). Cytogenetic study of Down syndrome cases in Nanning. *China* **31**:261–264.

Zubillaga A, Garrido I, Mugica J, Ansa R, Zabalza, Emparanza J, *et al*. (2006). Effect of vitamin D and calcium supplementation on bone turnover in institutionalized adults with Down's Syndrome. *Eur J Clin Nutr* **60**:605–609.

Holick MF (2006): *High Prevalence of Vitamin D inadequacy and implications for health*. Mayo Clin. Proc. **81**:353-73.

Broyles S, Katzmarzyk P, Srinivasan S, *et al*. (2010): *The pediatric obesity epidemic continues unabated in Bogalusa, Louisiana*. Pediatrics **125**:900-05.

Cacciari E, Milani S, Balsamo A, *et al*., (2006): Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yrs). Journal of Endocrinological Investigation **29**:581-93.

Devlin L and Morrison P (2004): *Accuracy of the clinical diagnosis of Down Syndrome*. The Ulster Medical Journal **73**:4-12.