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Fetuin A: Is a New Biomarker for Growth?

Esra Domur[1], Filiz Tubas[2], Vugar Ali Turksoy[3], Lütfiye Tutkun[4], Mujgan Ercan[5], Fatma İnci Arısoy[6]

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Abstract

Vitamin D deficiency is common in the World and Turkey due to changing lifestyles. The association with autoimmune diseases such as obesity, metabolic syndrome, diabetes, cardiovascular diseases and important diseases such as cancer has made it important to prevent vitamin D deficiency. Fetuin-A is a glycoprotein produced in the liver and it is effective in the inhibition of bone mineralization, insulin resistance, obesity and calcification in smooth muscle. In the study conducted, it was aimed to investigate the relationship between the two substances in growth retardation, considering that Vitamin D and Fetuin-A are acting through similar mechanisms. The study was conducted with 50 healthy children with growth retardation. In short patients and underweight patients, vitamin D levels were found to be lower than control group (respectively p = 0,011 p = 0,036). As vitamin D value increased one unit, it was found that the risk of growth retardation decreased by 1,071 times (p = 0,21). Fetuin-A levels were higher in those with growth retardation (p = 0,035). In the preschool group, fetuin-A was higher than middle childhood (p < 0,001). In short patients, fetuin-A levels were higher than control groups (p = 0,043). There was a poor correlation between vitamin D and fetuin-A levels in those with growth retardation (rho: 0,366 p = 0,009). Fetuin-A levels were higher in short children and there was a positive relationship between vitamin D and fetuin-A levels in those with growth retardation. Vitamin D levels were lower in those with growth retardation.

Keywords: Fetuin-A; Vitamin D; Growth retardation; Underweight, Short stature.

Authors Affiliation: [1,2,6]Department of Child Health and Diseases, Yozgat Bozok University Faculty of Medicine, Yozgat, Turkey. [3]Department of Public Health, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey. [4]Department of Nutrition and Dietetics, Faculty of Health Sciences, Gazi University, Ankara, Turkey. [5]Department of Biochemistry, Faculty of Medicine, Harran University, Sanliurfa, Turkey.

Corresponding Author: Vugar Ali Turksoy, Department of Public Health, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey.

E-mail: v.aliturksoy@bozok.edu.tr

Introduction

Low height and/or weight measurements of children who have not completed growth compared to their peers is defined as growth retardation.[1] Growth retardation is common in patients with malnutrition, food intake problems, absorption disorders, chronic diseases such as chronic lung disease and cardiovascular disease.[2] Growth and height of the child primarily depends on many variable factors such as normal bone structure, proper nutrition, tissue oxygenation, pH of the environment, hormones, environment

and additional diseases. The relationships between growth hormone (GH), thyroid hormones, insulin-like growth Factor-1 (IGF-1), sex hormones in the pubertal period and their receptors are effective in achieving normal height growth.[3] Although the main factor in the development of bone mass is the genetic structure, various factors such as activity, nutrition and lifestyle also affect the genetic structure. The most important factors that play a role in the genetic structure are collagen Type 1, Vitamin D receptors (VDR), IGF-1 and estrogen related genes.[4] The prevalence of growth retardation may vary depending on the definition of the term and the participant and therefore, it has been stated that it is between 1.3% and 20.9% in various sources.[5] Growth retardation may have many different causes, however irrespective of the reason, it is known that a child with growth retardation may have many mental, physical and psychological problems in the future.[2] Growth retardation in the world and in Turkey decreased over the years, though, it remains important because of the advanced stage effects.[1,6] Nutritional disorders and deficiency of vitamins and minerals are seen in children with growth retardation.[7] Although treatment and follow-up are important in the approach, early diagnosis and prevention efforts are more effective to reduce cost and comorbidity.

There are different parameters depending on the type of percentile curve used to express growth retardation. These are listed below:

1. Body mass index is below the 5th percentile
2. Height-for-age is below the 5th percentile
3. Weight-for-age is below the 5th percentile
4. Weight deceleration that crosses two major percentile lines on a growth chart

It is accepted that there is growth retardation in the presence of at least one of the mentioned criteria.[7]

Vitamin D is a fat-soluble vitamin and has been recognized as a hormone when its functions and structure are better understood.[8] It can be taken with diet, it can also be synthesized in the body by the effect of sun rays. Its main function is on calcium and phosphorus metabolism.[7] If the calcium level in the body is sufficient, calcium and phosphorus are absorbed from the intestines by the effect of 1.25 $(OH)_2D$ while bone mineralization is provided.[7] Vitamin D metabolism is tightly regulated by calcium, phosphorus, PTH serum levels, fibroblast growth factor 23 (FGF 23) and 1,25 $(OH)_2D_2$.[7] While vitamin D is necessary to maintain normal calcium levels in adults, calcium homeostasis and bone development are not dependent on vitamin D levels in the fetal period.[9] Dark skin color, insufficient sunlight, absorption disorders, the use of anticonvulsants and some drugs increase the risk of Vitamin D deficiency.[10] It is commonly seen in Turkey and worldwide.[11–13] Vitamin D deficiency is the most common cause of osteomalasia and if the bone has not completed its growth, rickets is seen and as a result the bone growth is disrupted.[7] In today's world lifestyles are changing in many ways and increased amount of time spent indoors reduces sun exposure.

However, when examined in more detail, it is understood that vitamin D deficiency causes problems clinically even without rickets. Vitamin D has been shown to be associated with autoimmune diseases such as cancer, metabolic syndrome, cardiovascular diseases and diabetes.[10]

To define Vitamin D status in healthy children and adolescents, the following standards of the 2016 Global Consensus Guidelines,[14] based on the measurement of 25 (OH) D serum concentrations, similar to the recommendations of the Pediatric Endocrine Society, are used.

- Vitamin D sufficiency: 20 to 100 ng/mL (50 to 250 nmol/L)
- Vitamin D insufficiency: 12 to 20 ng/mL (30 to 50 nmol/L)
- Vitamin D deficiency: <12 ng/mL (<30 nmol /L)

Fetuin-A is a glycoprotein produced mainly in the liver and is produced abundantly in multiple tissues during embryogenesis and the amount decreases after birth.[15–17] This suggests that Fetuin-A may be related to organ development and growth, and also acts as a negative acute phase reactant.[15] It is one of the most common non-collegenous proteins in bone and teeth.[18] It prevents calcification in smooth muscles.[19] In addition to being an ectopic calcification inhibitor, it has been shown to have many different functions.[15] Fetuin-A acts as an inhibitor of transforming growth factor-β (TGF-β), insulin-like growth factor (IGF) and hepatocyte growth factor [liver growth factor (HGF)].[20] With this aspect, it acts as a regulator in tissue regeneration and has roles in bone metabolism.[20,21] Metabolic syndrome has been associated with insulin resistance and diabetes.[22–24]

Fetuin-A plays a role in calcium and phosphate balance. Although its effect on bone formation and mineralization is not fully understood, it has strong affinity to hydroxyapatites and plays a role in bone formation.[25]

It was found that Fetuin-A level is inversely related to cardiovascular disease risk.[26] Fetuin-A deficient mice showed resistance to weight gain.[27] Only normal Fetuin-A levels are useful for humans.[28] However, not all functions of Fetuin-A have been fully elucidated.

The effects of Fetuin-A on bone metabolism and its relationship with metabolic syndrome and diabetes brings in mind vitamin D which has similar effects.[7,10,16,20,22,23,25,27] As a result of the literature search, no article examining Fetuin-A relationship with children with growth retardation was found.

The aim of this study was to evaluate the level of Fetuin-A in patients with growth retardation and to determine its relationship to weight or height, and to assess its association with Vitamin D, if any.

Materials and Methods

The study was started after the approval of the ethics committee of Bozok University Research and Application Hospital numbered 2017–04/02. The research was supported by Bozok University Scientific Research Projects Unit with the project number of 6602b-TF/17–106.

The study was conducted between February 2017 and May 2018 by informing the children and their families who applied to our outpatient clinic and fulfilling the inclusion criteria, and obtaining an informed consent form. Detailed medical history was taken and physical examinations of the subjects were performed and those who were in accordance with the study criteria after anthropometric measurements were included in the study. Participants were divided into two groups as healthy and growth retarded. Each group was divided into play-age children (1–6 years) and school-age children (7–11 years) according to age groups.

Study Group Acceptance Criteria:

1. Being between the ages of 1–11.
2. Height-for-age and weight-for-age measurements which are below 3 percentile according to Olcay Neyzi's percentile curves or height-for-age and weight-for-age measurements at -2SD and/or 2SD growth deviation (growth pause) during follow-up.
3. Having no chronic or acute disease in terms of genetic, metabolic, liver, congenital, cardiovascular, renal, respiratory or other systems.
4. Not taking medication for any reason
5. Having not taken vitamin D supplements in the last 3 months.
6. Voluntarily participating the study and signing the inform consent form.
7. Venous blood samples will be taken on the day of admission for any reason.

In the control group, the following conditions were requested: To have a 3–97 percentile curve according to the percentile curves of the same age range, to have no disease, not to use medication, not to use vitamin D in the last 3 months, voluntarily participating the study and having venous blood sample taken on the admission day.

The children included in the study were duly measured and weighed by trained personnel. Centimeters (cm) were used as units of length. Kilograms (kg) were used as a weighing unit. All of the measurements were marked on the growth charts prepared by Olcay Neyzi et al.[29] according to the age and gender. Afterwards, detailed anamnesis of the participants was taken according to their chronic or acute diseases, medications, nutritional history and family history and detailed physical examinations were performed.

Venous blood samples were taken to vacuum gel flat tubes for the tests required by the participants. No additional intervention was performed for the study purpose. On the same day the samples analyzed, each of the serum samples were taken into two eppendorf and frozen at –20°C. Samples were then transferred to Bozok University Science and Technology Application and Research Center Laboratory in the cold chain equipment and stored at –20°C up to the analysis time. Afterwards, the samples were thawed and Vitamin D and Fetuin-A levels were analyzed.

Olkowski et al. method[30] was used for Vitamin D analysis. Human (FETU-A) ELISA Kit (Sun Red, catalog number: 201-12-1387) was used for Fetuin-A analysis. Subjects were not given any special treatment for the study. However, necessary follow-up and treatments were performed according to the results of medical examination and accomplished tests.

Statistical Method

Data were evaluated by IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA) statistical package program. Descriptive statistics were given as number of units (*n*), percentage (%), median (M), first quartile (Q1) and third quartile (Q3) values. The normal distribution of the numerical variables was evaluated by the Shapiro Wilk normality test

and Q-Q plots. Mann-Whitney U test was used for comparison of two groups and Kruskal-Wallis test for more than two group comparisons. Dunn-Bonferroni test was used as a multiple comparison test if there was a difference in Kruskal Wallis test. The relationships between numerical variables were evaluated by Spearman correlation analysis. The relationship between categorical variables was examined by the exact method of Pearson chi-square test in 2 × 2 and rxc tables. If the Pearson chi-square test result is significant in the rxc tables, two proportion *z* test with Bonferroni correction were used as the sub-analysis. Backward elimination Wald method of binary logistic analysis was used to determine the factors affecting the dependent categorical variable (study control). $p < 0.05$ was considered statistically significant.

Results

The clinical and demographic characteristics of the patient and control groups are shown in Table 1.

In the study, no difference was found between the groups in terms of age, age group and gender distributions. Fetuin-A levels were found to be statistically higher in the study group than the control group ($p = 0.035$). Vitamin D was found higher in control group ($p = 0.01$). When the percentile evaluation of the children was made according to their weight, it was found that 38% of the control group were between 10 and 25 percentile and 28% were between 25 and 50 percentile and 66% of the patients in the study group were under 3 percentile. When the percentile was evaluated according to height, it was also seen that 26% of the control group were between 10 and 25 percentile and 24% were between 25 and 50 percentile, while 64% of the patients in the study group were below 3 percentile.

When the patients in the study group were classified according to their gender and age groups, they were similar in terms of height and weight percentiles ($p > 0.05$).

The subjects in the study group were grouped according to their anthropometric measurements and gender. Those whose weight is below 3 percentile are grouped as thin, those who were under 3 percentile in length were short, and those who were both thin and short were classified as thin + short (Table 2).

Calcium, phosphorus and ALP levels were found to be within normal range in both study and control

Table 1: Demographic and clinical characteristics of participants

Gender	Groups		*p*
	Healthy Group *n* (%)	Study Group *n* (%)	
Boy	28 (56.0)	34 (68.0)	0.303
Girl	22 (44.0)	16 (32.0)	
School-age	21 (42.0)	15 (30.0)	0.298
Play-age	29 (58.0)	35 (70.0)	
Age (years)	5.5 (3.0 – 8.3)	5.0 (2.0 – 8.0)	0.289
Fetuin-A (mg/L)	1132.14 (793.31 – 1265.95)	1240.11 (920.97 – 1317.85)	0.035
Vitamin D (ng/mL)	19.63 (15.52 – 27.53)	16.53 (13.17 – 19.49)	0.01

Table 2: Classification of children in the study group according to anthropometric measurements and age groups

	Age Groups		*p*
	School	Play	
	n (%)	*n* (%)	
Thinness status			
Normal	6 (40.0)	11 (31.4)	0.746
Thin	9 (60.0)	24 (68.6)	
Shortness status			
Normal	4 (26.7)	14 (40.0)	0.523
Short	11 (73.3)	21 (60.0)	
Thinness and shortness			
Normal	9 (60.0)	23 (65.7)	0.754
Thin and short	6 (40.0)	12 (34.3)	

groups (Respectively; Calcium mean value: 9.8 mg/dL; Phosphorus mean value: 4.6 mg/dL; ALP mean value: 163.1 IU/L; Calcium mean value: 10.8 mg/dL; Phosphorus mean value: 4.1 mg/dL; ALP mean value: 175.1 IU/L). In the study and control groups, vitamin D level was found to be 17.69 (14.21–23.41) ng/mL in males and 16.58 (12.86–22.19) ng/mL in girls (p = 0.28). Moreover, Fetuin-A levels were found to be 1218.21 (881.19–1311.06) mg/L in males and 1195.20 (824.11–1273.53) mg/L (p = 0.23) in girls.

Vitamin D deficiency was found in 18% and vitamin D insufficiency was found in 49% of all participants. Furthermore, vitamin D deficiency was found in 22% and vitamin D insufficiency was found in 58% of the study group.

When grouping according to vitamin D levels (deficiency, insufficiency, sufficiency), no difference was found in Fetuin-A levels according to groups (p > 0.05) (Table 3). When the control and study groups were grouped according to vitamin D levels, no difference were found in Fetuin-A levels according to the groups (respectively p = 0.823, p = 0.067).

A weak positive relationship was found between Fetuin-A and vitamin D only in the study group (p: 0.009 ρ: 0.366). Further statistical analysis showed that as vitamin D level increases by one unit, growth retardation decreases by 1.071 (1/0.934) times. No such association was found with Fetuin-A.

Table 3: Vitamin D and Fetuin-A levels by groups

	Control School	**Study School**	**Control Play**	**Study Play**	
	***n* = 21**	***n* = 15**	***n* = 29**	***n* = 35**	***p***
	***M* (Q_1 – Q_3)**	***M* (Q_1 – Q_3)**	***M* (Q_1 – Q_3)**	***M* (Q_1 – Q_3)**	
Fetuin-A (mg/L)	831.52 (724.56–1043.40)[a]	839.94 (660.60–1235.24)[a]	1227.03 (1114.32–1274.19)[b]	1301.17 (1190.67–1334.28)[b]	<0.001
Vitamin D (ng/mL)	18.62 (15.79–29.22)[a]	14.13 (11.59–16.54)[b]	20.3 (13.70–24.78)[a]	17.44 (14.25–22.25)[ab]	0.005

The superscripts *a, b* indicate differences between groups. Groups with the same letters are similar.

Fetuin-A levels were lower in school groups than in play groups (p < 0.001). Vitamin D levels were found to be lower in the school group compared to the other groups (p = 0.005). There was no statistical difference found in vitamin D levels in other groups. Vitamin D and Fetuin-A levels according to thinness and shortness are shown in Table 4.

Vitamin D levels of the thin ones were lower than normal ones (p = 0.036). Fetuin levels were higher in the short group (p = 0.043) and vitamin D levels were higher in the normal group (p = 0.011). Fetuin-A levels were found to be statistically different in play and school groups (p < 0.001). The play group Fetuin-A levels were found to be higher in the shorter patients compared to the school groups (p < 0.001). In comparison with school-normal group and play-normal group, vitamin D levels were found to be lower in the school-short group (p = 0.019). Moreover, Fetuin-A levels in the play group were higher than school groups (p < 0.001).

Table 4: Fetuin-A and Vitamin D levels according to age group and thinness

	School Normal	**School Short**	**School Thin**	**School Thin/ Short**	**Play Normal**	**Play Short**	**Play Thin/ Short**	**Play Thin**
	***n* = 21**	***n* = 11**	***n* = 9**	***n* = 6**	***n* = 29**	***n* = 21**	***n* = 12**	***n* = 24**
	M (Q_1 – Q_3)	**M (Q_1 – Q_3)**	**M (Q_1 – Q_3)**	**M (Q_1 – Q_3)**	**M (Q_1 – Q_3)**	**M (Q_1 – Q_3)**	**M (Q_1 – Q_3)**	**M (Q_1 – Q_3)**
Fetuin-A (mg/L)	831.52 (724.56 – 1043.40)	885.85 (768.92 – 1244.47)	885.85 (698.78 – 1214.08)	909.26 (785.36 – 1252.36)	1227.03 (1114.32 – 1274.19)	1296.19 (1201.26 – 1336.23)	1292.44 (1185.73 – 1337.10)	1292.44 (1174.90-1334.43)
Vitamin D (ng/mL)	18.62 (15.79 – 29.22)	13.95 (11.59 – 16.63)	14.24 (12.97 – 16.73)	15.33 (13.46 – 17.09)	20.3 (13.70 – 24.78)	16.94 (13.73 – 22.27)	17.19 (12.20 – 23.32)	17.75 (13.16 – 20.58)

Discussion

Growth retardation remains one of the serious problems of childhood. Although many factors cause growth retardation, malnutrition and arbsorption disorders are prominent.[1] Early diagnosis and treatment is important because of complications that may cause in the future.

In this study, when the weight was evaluated according to height in children who were shorter for their age, there was no child whose weight was below 3 percentile. This was attributed to chronic malnutrition of the patients participating in the study.

In previous studies, Fetuin-A levels in children were found to be 0.52 ± 0.009 mg/mL in children between the ages of 6 and 18 by Van Summeran et al.,[31] 0.3 (0.21–0.52) mg/mL in children between the ages of 5–12 years by Marhaug et al.[15] and 0.22–0.70 g/L (0.46 + 0.24 g/L) in healthy children by Wigger et al.[32]

In this study, it was found in [1132.14 (793.31–1265.95)] mg/L range in healthy subjects and in [1240.11 (920.97–1317.85)] mg/L range in patients with growth retardation. Fetuin-A levels were found to be higher in both groups in comparison to levels reported in some adults and pediatric studies of the literature.[15,24,31-34]

Similar to other studies in the literature, no difference was found between the genders.[32,34] Although the reason for these high levels could not be fully explained, it was thought to be related to race, geography or environmental factors. In some studies, a slight increase with age has been proven and in some other studies a decrease by time after prenatal period has been proven.[15,17,34,35] Most of these studies were done in people with comorbidities. When evaluated with this aspect, no comparative study on growth need was found in the literature. These variable data related to age suggest that release of Fetuin-A is affected by more than one factor and that there is no constant release rate of Fetuin-A. As a result of statistical analysis, Fetuin-A levels and gender and weight were not correlated in this study. When the patients were classified as play-age and school-age according to their growth stages, we observed that Fetuin-A levels were lower than that of play-age children during the school period where growth is slower ($p < 0.001$).

Fetuin-A is abundant in fetal bovine serum, fetal blood and tissues and this suggest that Fetuin-A may play a more general role in organ development.[18,19] In a study, when preterms, very low birth weight infants, infants, school-age children and adolescents were evaluated, the highest Fetuin-A levels were highest in preterms born at 24–30 weeks of gestation. When preterms, very low birth weight infants, infants, school-age children and adolescents were evaluated by a study, the highest Fetuin-A levels were measured in preterms born at the 24–30 weeks of gestation. Afterwards, Fetuin-A levels were found to be decreased and reached adult levels and this decrease was found to be related to biological age rather than chronological age.[17] In a study with sheep, a decrease with age was reported after high prenatal serum Fetuin-A.[35] These data support that Fetuin-A is high in organisms in need of growth and their concentration depends on biological age. In this case, Fetuin-A can be assumed to stimulate growth.[17] In our study, serum Fetuin-A levels were found to be higher in children with short stature than healthy control group ($p = 0.035$). This elevation in serum Fetuin-A level was thought to be related to the growth need. It should be remembered that children in our study group were not able to achieve age-appropriate growth and could not yet complete their chronological age-appropriate growth compared to their peers. As a result of this idea, the Fetuin-A level should be expected to increase during the periods when the biological age of the child who has not yet completed the growth remains behind the chronological age or when the catch-up growth is required (such as the catch-up growth of the premature). When the need for growth is reduced, namely, when the child reaches the characteristics of its chronological age, serum Fetuin-A levels should decrease to reach a stable age-appropriate level. In accordance with this study, Shroff et al.[34] showed that Fetuin-A levels were lower in healthy children aged between the ages of 12 and 18 who are longer than 50 percentile compared to their peers.

Fetuin-A should always remain stable in the body to a certain extent, given its non-bone functions. In a study conducted by Topsakal et al.[36] with 37 patients with acromegaly and 30 healthy participants, Fetuin-A levels were found to be very high and significant statistically in acromegalic patients. In addition, IGF-1 levels were found to be correlated with Fetuin-A levels. This study supports that Fetuin-A increases growth. Moreover, increase for any reason after the closure of the epiphyseal plate gives an impression that it may be related to pathological growth.

Newborns with intrauterine growth restriction (IUGR) were found to have defective glycosylation

of Fetuin-A.[37] However, concentrations of total Fetuin-A were found to be similar in healthy term newborns with growth restriction.[38] When these data are evaluated together with high fetuin levels in premature babies, it is suggested that the presence of Fetuin-A is not sufficient for proper growth and that its structure and receptor relations should be normal. In our study, the reason for finding high Fetuin-A levels in short participants might be the fact that the structure of Fetuin-A is not normal. Since the Fetuin-A structure could not be studied in this study, it was not possible to clarify this. Further studies are needed to confirm the possibility.

It has been proposed in a study conducted by Hausler et al.[17] that decrease in Fetuin-A serum concentrations to adult levels after intrauterine life and the presence of similar low Fetuin-A concentrations from early infancy to adulthood was not associated with a decrease in Fetuin-A synthesis. On the contrary, it has been suggested that Fetuin-A as a mineral chaperone is required and consumed during active skeletal mineralization and accumulate in the bone, as shown in an animal model.[25] The fact that Fetuin-A levels in shorter children are higher than healthy children can be evaluated as the accumulation of Fetuin-A in bone.

In contrast to high bone mass in healthy children and the inclusion of Fetuin-A in bone formation, it can be assumed that Fetuin-A is used less in short children because of its low bone mass compared to their peers and therefore serum Fetuin-A levels are higher. While Fetuin-A decreases in Paget's disease, the increase in Fetuin-A in osteogenesis imperfecta may be related to consumption of Fetuin-A, but may also be related to the need for growth and mineralization as discussed earlier.[39,40] However, this theory is based on the assumption that Fetuin-A release is relatively constant, although it explains the gradual decrease in Fetuin-A levels with age. Increased secretion in patients with acromegaly or changes in Fetuin-A levels in patients with osteoporosis indicate that Fetuin-A is not at a constant release rate and that the release rate may change.[36,41] Studies which are showing that the Fetuin-A levels increases with age contradicts the idea that Fetuin-A levels decreases because it accumulates in bone.[25] The risk of cardiovascular disease also rises as age increases.[42,43] However, the fact that Fetuin-A is lower at a younger age and increases with age also contradicts this information. Because low-level Fetuin-A will reduce the inhibition capacity of intravascular calcification, the risk of cardiovascular disease should increase as in the study of Ix et al.[26] It was found that the femurs of the mice whose Fetuin-A gene was genetically defected were extend more slowly between 3 and 18 months and especially the femur, one of the long bones, was found to be severely stunted. Bone composition, mineral and collagen properties of cortical bone were not affected by the absence of Fetuin-A. Mineralization of premature growth plate resulted in shortening of femoral length. In this context, it has been argued that Fetuin-A is a requirement for appropriate long bone growth, at least in mice.[16] This information supports the fact that Fetuin-A is not necessary for bone calcium accumulation and that mineralization occurs even if Fetuin-A is not present and that the effect of Fetuin-A on mineralization is more on stature. In addition to studies advocating that Fetuin-A should be increased in order to accelerate mineral formation *in vitro* and support collagen calcification, there are studies advocating that Fetuin-A should be low in terms of contributing to the procalcial environment for bone growth.[15,44]

Mathew's et al.[27] performed Fetuin-A gene ablation in mice causing complete deficiency of Fetuin-A and as a result of the study, it was found that mice did not gain weight despite fat feeding. Thinness can be expected in people with Fetuin-A deficiency due to this study, but complete deficiency of Fetuin-A has not been demonstrated in humans. Increased Fetuin-A levels have been associated with obesity.[22,45] In some studies, no correlation was found between Fetuin-A serum concentration and BMI-SDS.[32,46] Low serum Fetuin-A has been reported in infected and malnourished children.[21] These contradictory data indicate that the relationship between weight and Fetuin-A is not fully understood. In this study, we found that Fetuin-A was not associated with weight in thin or healthy children ($p = 0.064$). However, it should be remembered that the participants did not have heavy malnutrition.

In addition, vitamin D levels did not differ according to gender. Vitamin D levels were significantly lower in the group with growth retardation than healthy children ($p = 0.01$). Vitamin D levels were lower in both thin ($p = 0.036$) and short ($p = 0.011$) group than in the control group. In a study of mice examining inflammatory bowel diseases, IL-10 deficiency was induced in mice and a group of mice were specifically raised to lack vitamin D. It has been observed that mice with vitamin D deficiency started to eat less when

they were 9 weeks old and afterwards started lose weight rapidly. Control group mice with vitamin D deficiency were grew slower than vitamin D-sufficient/IL-10 deficient mice, but there was not found significant difference between the two groups at the week of 12.[47] Vitamin D deficient group was observed to grow more slowly but in the absence of additional disease, it was finally reached the target. Although it is not correct to adapt this information directly to human, we have found that vitamin D levels were lower in pediatric patients with growth retardation, it suggests that there may be a similar mechanism exist in humans. Although there are studies showing that there is no relationship between vitamin D and body mass index or weight, studies on weight gain and vitamin D in humans are usually related to obesity. In addition, there are no studies examining thinness and vitamin D in children.[48]

The group with the lowest vitamin D levels was school-age children with growth retardation. In this age group, increased time spent indoors such as schools and homes and increased veiling clothing style due to the region where we live may have caused vitamin D levels to decline as a result of decreased vitamin D synthesis in the skin.

Considering the relationship with calcium and studies on bone mineralization, it can be thought that vitamin D and Fetuin-A together affect bone mineralization. This suggests that vitamin D and Fetuin-A may be related. Both vitamin D and Fetuin-A have insulin resistance effect and are associated with obesity.[22,49] On the other hand, there are no studies in the literature that can clearly explain the relationship between the two. Hovewer, it has been shown in several studies that vitamin D administration affects Fetuin-A levels in animals and humans.[46,50,51] However, the possible outcome of interactions of Fetuin-A and vitamin D on bone mass is currently not fully known.

In a study of 112 children with chronic kidney disease, no correlation was found between Fetuin-A levels and vitamin D levels. In the same study, the annual cumulative dose of calcitriol associated with weight and a relationship between calcium and Fetuin-A was found.[46] It has been shown that 1.25 (OH) vitamin D levels correlate significantly positive with serum Fetuin-A in adults not receiving dialysis treatment with diabetic nephropathy and coronary artery calcification.[50] Moreover, in adult dialysis patients with secondary hyperparathyroidism, calcitriol has been shown not only to suppress PTH but also to stimulate serum Fetuin-A levels.[50] Keskin et al.[52] showed that Fetuin-A levels decreased after parathyroidectomy and Santos et al.[53] showed that vitamin D and Fetuin-A increased. Regardless of the triggering event, its effect on calcium homeostasis requires Fetuin-A, an important calcification inhibitor. Fetuin-A is required in order to prevent the formation of hydroxyapatite crystals and calcium chelation.[25] The effect of vitamin D treatment on Fetuin-A is partially achieved with serum calcium.[46] Nimitphong[54] showed that the relationship between Fetuin-A and bone mass varies according to DBP genotype and this effect is independent of vitamin D status. However, this observation requires further approval. In the study, the mean values of calcium were found to be normal in healthy and growth retarded group. When Fetuin-A levels were compared according to vitamin D levels in children with growth retardation, this difference was not statistically significant although there were higher levels of Fetuin-A in vitamin D-sufficient group compared to the other two groups but it was close to the level of significance (p = 0.067). The difference is considered to be significant if the number of samples is increased. In the study, a weak positive correlation was found between Fetuin-A and vitamin D only in the study group (p = 0.009 ρ: 0.366). There was no relationship between Fetuin-A and vitamin D in healthy group (p = 0.97). In view of the fact that this group is children who have not completed their age-appropriate growth, it is expected that Fetuin-A will increase in parallel with vitamin D in order to increase the calcium absorption of vitamin D and to allow the deposition of increased calcium to the bone.

Conclusion

To our knowledge, this is the first study examining the relationship between growth retardation and Fetuin-A. Patients with growth retardation compared with healthy patients, a correlation was found between vitamin D and Fetuin-A levels. As a conclusion, vitamin D and Fetuin-A levels were found to be correlated with patients with growth retardation compared to healthy patients but further studies are needed to explain this relationship.

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