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The effect of 2000 ıu/day vitamin D supplementation on insulin resistance and cardiovascular risk parameters in vitamin D deficient obese adolescents

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The aim of this study was to determine the vitamin D deficiency prevalence in obese adolescents and to investigate the effect of vitamin D supplementation on insulin resistance and cardiovascular risk parameters in obese adolescents with vitamin D deficiency. Ninety-six obese adolescents aged 10-18 years were divided in 2 groups according to their vitamin D levels: Deficient group (<12ng/ml) and sufficient group (≥12ng/ml). All patients in the vitamin D deficiency group were recommended 2000IU/day vitamin D supplementation. Fifty four (56.3%) patients had vitamin D deficiency. The only difference between the two groups was PTH level which was higher in the vitamin D deficiency group. Vitamin D reached sufficient levels in 22 (95.6%) out of the 23 patients with the 3 month supplementation of 2000 IU/day vitamin D. There was a significant decrease in weight Standard Deviation Score (SDS), Body Mass Index (BMI) SDS, hip circumference, total cholesterol, LDL, HbA1c, AST, PTH and interleukin-6 while no significant change was seen in measurements of glucose, insulin, HOMA-IR, C-peptide and the rate of metabolic syndrome. There were decreases in levels of total cholesterol and LDL with vitamin D treatment, while there was no significant change in insulin resistance. Vitamin D reduced interleukin-6 levels by its antiinflammatory effect.

Key words: cardiovasculer risk, insulin resistance, obesity, vitamin D.

Obesity is a public health problem that leads to multiple complications in children such as hyperinsulinemia, insulin resistance (IR), type 2 diabetes mellitus (DM), hypertension, hyperlipidemia, atherosclerosis and cardiovascular disease. Obesity is placed as a major risk group for vitamin D deficiency because of decreased vitamin D bioefficiency secondary to vitamin D sequestration in subcutaneous fat tissue.[1,2] Serum levels of 25(OH)D vitamin in obese population has been found to be lower in many studies.[3-5] Recent studies have shown that vitamin D has many functions in the body, it acts more likely as a hormone than a vitamin since it affects different cites other than where it has been synthesized and vitamin D defiency is related to many diseases.[6,7] In studies investigating the role of vitamin D in type 2 DM etiology, it's been found that vitamin D supplementation results in increased insulin sensitivity, rearrangement of beta cell function and normalization of impaired glucose values.[8] It has been revealed in both animal and in vitro studies that vitamin D increases both secretion and release of insulin.[9,10] Vitamin D also has anti-atherosclerotic effects by impeding macrophages to turn into foam cells and suppressing the inflammation.[11,12]

Determination of both the level of vitamin D and the rate of vitamin D deficiency in obese adolescents; evaluation of whether there are differences in IR and cardiovascular risk

parameters between vitamin D sufficient and deficient groups and determination of whether there would be any changes in IR and cardiovascular risk parameters in the vitamin D deficient group after vitamin D supplementation were aimed in this study.

Material and Methods

Following ethics committee approval (approval no: 60/2015), 10-18 year old adolescent cases with body mass index (BMI) ≥ 95 percentile admitted to pediatric endocrinology clinic between November 2015 and May 2016 were included in the study. All parents gave written informed consent before participation. Patients with chronic systemic diseases, continuous drug users and those who had obesity other than exogenous obesity (due to syndromic or endocrinologic reasons) were excluded from the study. Gestational age, birth weights in all patients and presence of obesity, type-2 DM and atherosclerotic CVD in first and second degree family members of all cases were recorded. Height (with Harpenden stadiometer) and weight (with SECA brand weight meter) measurements were done by the same doctor (MEB). BMI was calculated by weight (in kilograms) over height squared (in meters).[13] Standard deviation score (SDS) was calculated for height, weight and BMI. Waist circumference was measured when the position of the patient was standing erect with relaxed abdominal muscles and arms next to the body and feet united, between the lowest rib and the top of iliac crest over the umbilicus horizontally via unflexible tape. Waist circumferences were evaluated by percentile curves created for Turkish children.[14] Hip circumference measurement was done using an unflexible tape ensuring its horizontal position, placed around the point with the maximum circumference over the buttocks. Blood pressure values of all cases were measured with age and arm length appropriate manual blood pressure cuff. Blood pressure values were evaluated according to blood pressure percentile curves.[15] Puberty stages were determined according to Tanner-Marshall criteria.[16]

All cases had detailed physical examinations and acanthosis nigricans status of the cases was recorded. Plasma glucose, insulin, lipid profile, HbA1c, C-peptide, 25(OH)D, calcium, phosphorous, ALP, PTH, hs-CRP and IL-6 levels were of all cases measured after a 10-hour fasting period. Glucose, HbA1c, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride levels were measured with Architect C16000 oto-analyzer system, insulin levels were measured by the chemiluminescence method (Advia Centaur X-P). For evaluating IR, HOMA-IR value was calculated by the formula: "fasting insulin (mIU/ml) x fasting glucose (mmol/lt) / 22.5". HOMA-IR>2.6 for pre-pubertal boys, HOMA-IR>5.2 for pubertal boys, HOMA-IR>2.2 for pre-pubertal girls and HOMA-IR>3.8 for pubertal girls were described as IR.[17] Metabolic syndrome (MS) was determined according to International Diabetes Federation (IDF) criteria for children.[18] C-peptide value was measured with the device Abbott I-2000. The values between 0.9 and 7.1 were accepted as normative data. Calcium, phosphorous, ALP and PTH values were measured with the device Synchron DxC 800 pro Coulter Beckman. Normal range for PTH was accepted as 11-67 pg/ml. Serum 25(OH)D concentrations were measured at hospital laboratory by chemiluminescence method with LC-MS/MS. According to vitamin D levels patients were divided in 2 groups. Cases with vitamin D <12 ng/ml were included to "Vitamin D deficient group" and cases with vitamin D ≥12 ng/ml were included to "Vitamin D sufficient group".[19] A 2000 IU/day vitamin D treatment was prescribed to vitamin D deficient cases. Patients who accepted the treatment and used it regularly for 3 months were referred to as the "Treatment group". The treatment group was given a diet program which had a 10% reduced calorie intake with sufficient daily calcium appropriate for age.[20] Hs-CRP was measured by florescence immunoassay method with the device Fine Care, IL-6 was measured by chemiluminescence method with the device Siemens Immune Light 2000.

All analyses were conducted with SPSS version 22.0 for Windows (IBM SPSS version 22.0; IBM, New York, N.Y., USA). Numerical variables were expressed as mean ± standard deviation or median (min-max). Categorical variables were expressed by numbers and

percentages. Paired t-test was used to compare variables with a normal distribution, Wilcoxon rank test was used to compare variables with a non-normal distribution. Differences between two independent groups in terms of numerical variables were evaluated with a t-test in the cases that met the assumptions of parametric testing. In those cases that did not meet the assumptions of parametric testing, Mann-Whitney U test was used. Chi-square test was used to determine whether there were any differences between the groups in terms of categorical variables. Variations in groups were identified with Wilcoxon test or Friedman test. A p value of less than 0.05 was set for statistical significance.

Results

A total of 96 obese adolescents admitted to pediatric endocrinology department between November 2015 and May 2016 were enrolled in the study. There were 59 (60.8%) girls and 38 (39.2%) boys. The mean age of all cases was 13.17 ± 2.4 years. Fifty-four (56.2%) cases had vitamin D deficiency while the other fourty-two (43.8%) cases had a sufficient vitamin D level. Demographic data for all groups are given at Table I. Vitamin D deficient and sufficient groups were similar in terms of the mean age, gender, gestational age, birth weight according to gestational age and family histories for obesity, diabetes mellitus and cardiovascular diseases. Anthropometric measures, pubertal stages and acanthosis nigricans status for all groups are given in Table II. There was no difference between Vitamin D deficient and sufficient groups in terms of height SDS, weight SDS, BMI SDS, waist circumference, hip circumference, puberty stages and acanthosis nigricans status. Calcium, vitamin D metabolism, glucose metabolism and cardiovascular risk parameters for all groups are given in Table III. There was no significant difference between Vitamin D deficient and sufficient groups in terms of calcium, phosphorus, alkaline phosphatase, fasting plasma glucose, fasting insulin, HOMA-IR, fasting C-peptide, HbA1c, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL cholesterol, triglyceride, hs-CRP, IL-6 and AST. The mean vitamin D level was 8.1 ± 3.3 (4-11.4) ng/ml in the vitamin D deficient group and 20.7 ± 6.2 (13.5-39.9) ng/ml in the vitamin D sufficient group. PTH levels were higher in the vitamin D deficient group in comparison to the vitamin D sufficient group (p=0.015). Eleven cases (20.3%) from the vitamin D deficient group and 13 cases (30.9%) from the vitamin D sufficient group had metabolic syndrome (MS). There was no statistically significant difference between groups in term of MS frequency.

Fifty-four cases with vitamin D deficiency were advised to use 2000 IU/day oral vitamin D for 3 months. Twelve cases (22.2%) refused the treatment. Four (9.5%) of 42 cases accepting the treatment had side effects (3 had skin eruptions, 1 had gastrointestinal intolerance)

Table I. Demographic Characteristics of Study Population.

	Both Groups n=96	Vitamin D Deficient Group n= 54	Vitamin D Sufficient Group n=42	p
Age (years)	13.1 ± 2.4	13.3 ± 2.4	12.9 ± 2.3	0.46
Sex (F/M)	59/37	37 / 17	22 / 20	0.107
Gestational age (Term/Preterm)	85/11	49/5	36/6	0.52
Birth weight	3272.2 ± 706.1	3244.9 ± 764.3	3307.3 ± 630.8	0.42
AGA/LGA/SGA	78/13/5	40/11/3	38/2/2	0.80
Family obesity	64 (66.7%)	38 (70.4%)	26 (61.9%)	0.38
Family diabetes	58 (60.4%)	33 (61.1%)	25 (59.5%)	0.87
Family ASHD	39 (40.6%)	21 (38.9%)	18 (42.9%)	0.69

F/M: Female/Male, AGA: appropriate for gestational age, LGA: large for gestational age, SGA: small for gestational age, ASHD: arteriosclerotic heart disease

Table II. Antropometrics Characteristics,Pubertal Stages and Acantosis Nigricans Ratio of Study Population.

	Both Groups n=96	Vitamin D Deficient Group n= 54	Vitamin D Sufficient Group n=42	p
Height (cm)	155.9 ± 8.8	156.1 ± 9.1	155.7 ± 8.5	0.84
Height SDS	0.29 ± 1.14	0.15 ± 1.09	0.47 ± 1.18	0.15
Weight (kg)	76.9 ± 16.2	78.4 ± 16.4	74.9 ± 15.9	0.29
Weight SDS	2.74 ±0.95	2.79 ±1.0	2.67 ± 0.88	0.82
BMI	32.1 ± 11.3	31.5 ± 4.2	32.9 ± 16.5	0.53
BMI SDS	2.67 ± 0.55	2.75 ± 0.61	2.55 ± 0.44	0.19
Waist circumference (cm)	97.2 ± 11.6	95.8 ± 11.6	98.9 ± 11.5	0.21
Hip circumference (cm)	108.2 ± 10.7	108.9 ± 10.6	107.4 ± 10.9	0.50
Pubertal stage (Tanner 1/2/3/4/5)	9/20 /12/12/43	4 / 7 / 6 /11/ 26	5 / 13 / 6 /1/17	0.029
Acantosis nigricans	46 (47.9%)	25 (46.3%)	21 (50%)	0.71

BMI: body mass index, SDS: standart deviation score

Table III. Laboratory Characteristics of Study Population.

	Both Groups n=96	Vitamin D Deficient Group n= 54	Vitamin D Sufficient Group n=42	p
Calcium (mg/dl)	9.74 ± 0.35	9.71 ± 0.34	9.78 ± 0.37	0.36
Phosphorus (mg/dl)	4.61 ± 0.66	4.61 ± 0.69	4.61 ± 0.62	0.97
Alkaline phosphatase (U/L)	187.3 ± 93.1	170.8 ± 86.6	208.6 ± 97.8	0.08
25(OH) vit D (ng/ml)	13.6 ± 7.9	8.1 ± 3.3	20.7 ± 6.07	0.0001
Parathormon (pg/ml)	62.0 ± 29.7	70.3 ± 30.8	51.4 ± 24.8	0.02
Fasting glucose (mg/dl)	89.3 ± 6.0	89.5 ± 6.2	89.0 ± 5.8	0.81
Fasting insulin (mIU/ml)	25.0 ± 21.6	24.5 ± 13.2	25.6 ± 29.3	0.27
HOMA-IR	5.56 ± 5.1	5.4 ± 3.1	5.6 ± 6.9	0.21
C peptid (ng/ml)	2.98 ± 1.84	2.72 ± 1.3	3.3 ± 2.33	0.76
HbA1c (%)	5.1 ± 0.26	5.1 ± 0.3	5.1 ± 0.19	0.32
Systolic blood pressure (mmHg)	117.7 ± 12.5	118.1 ± 14.0	117.2 ± 10.5	0.97
Diastolic blood pressure (mmHg)	75.7 ± 8.9	75.6 ± 7.8	75.9 ± 10.1	0.81
Total cholesterol (mg/dl)	166.1 ± 29.4	169.7 ± 31.9	161.5 ± 25.6	0.17
LDL cholesterol (mg/dl)	99.9 ± 27.6	103.6 ± 30.1	95.1 ± 23.6	0.13
HDL cholesterol (mg/dl)	44.1 ± 10.8	44.3 ± 11.8	43.9 ± 9.6	0.86
Triglyceride (mg/dl)	125.2 ± 53.4	120.3 ± 49.2	131.6 ± 58.2	0.51
Sensitive CRP (mg/dl)	0.44 ± 0.98	0.35 ± 0.33	0.55 ± 1.43	0.56
Interleukin 6 (pg/ml)	3.31 ± 1.88	3.07 ± 1.58	3.63 ± 2.21	0.30
AST (U/L)	24.7 ± 29.7	27.2 ± 38.9	21.5 ± 7.9	0.88
ALT (U/L)	26.9 ± 41.0	30.8 ± 52.9	21.9 ± 15.3	0.40

HOMA-IR: homeostatic model assessment-insülin resistance, HbA1c: hemoglobin A1c, LDL: low density lipoprotein, HDL: high density lipoprotein, CRP: C reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase

Table IV. Comparison of Antropometric, Clinic and Laboratory Characteristics Before and After Vitamin D Treatment in Vitamin D Deficiency Group.

	Before treatment	After treatment	P
Height (cm)	155.8 ± 7.6	157.1 ± 7.3	0.0001
Height SDS	0.10 ± 1.32	0.18 ± 1.26	0.56
Weight (kg)	74.5 ± 13.3	73.4 ± 13.2	0.09
Weight SDS	2.48 ± 0.95	2.44 ± 2.63	0.03
BMI	30.1 ± 4.21	29.6 ± 3.84	0.27
BMI SDS	2.58 ± 0.67	2.34 ± 0.68	0.008
Waist circumference (cm)	93.6 ± 10.4	91.1 ± 8.32	0.16
Hip circumference (cm)	107.4± 10.4	104.7 ± 10.5	0.02
Calcium (mg/dl)	9.7 ± 0.30	9.73 ± 0.31	0.31
Phosphorus (mg/dl)	4.5 ± 0.76	4.37 ± 0.65	0.37
Alkaline phosphatase (U/L)	176.5 ± 81.6	180.7 ± 84.9	0.68
25(OH) vit D (ng/ml)	7.5 ± 2.7	23.4 ± 9.7	0.0001
Parathormon (pg/ml)	69.3 ± 22.0	56.3 ± 18.4	0.016
Fasting glucose (mg/dl)	89.0 ± 6.4	87.4 ± 7.2	0.24
Fasting insulin (mIU/ml)	20.8 ± 7.9	20.3 ± 9.6	0.77
HOMA-IR	4.6 ± 1.8	4.4 ± 2.2	0.67
C peptid (ng/ml)	2.62 ± 1.65	2.57 ± 1.24	0.91
HbA1c (%)	5.05 ± 0.33	5.1±0.27	0.049
Systolic blood pressure (mmHg)	116.3 ± 7.5	115.4± 11.3	0.69
Diastolic blood pressure (mmHg)	75.8 ± 7.4	74.3 ± 8.4	0.14
Total cholesterol (mg/dl)	168.6 ± 29.6	157.7 ± 28.6	0.018
LDL cholesterol (mg/dl)	103.1 ± 31.0	93.9 ± 29.0	0.004
HDL cholesterol (mg/dl)	46.4 ± 11.5	45.5 ± 7.3	0.55
Triglyceride (mg/dl)	118.0 ± 47.0	114.5 ± 64.5	0.20
Sensitive CRP (mg/dl)	0.34 ± 0.43	0.25 ± 0.19	0.23
Interleukin 6 (pg/ml)	3.05 ± 1.57	2.17 ± 0.86	0.04
AST (U/L)	21.2 ± 5.2	20.0 ± 4.4	0.006
ALT (U/L)	20.8 ± 7.3	20.04 ± 7.2	0.34

BMI: body mass index, SDS: standart deviation score, HOMA-IR: homeostatic model assessmentinsülin resistance, HBA1c: hemoglobin A1c, LDL: low density lipoprotein, HDL: high density lipoprotein, CRP: C reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase

after which treatment was stopped. Nine cases (21.4%) didn't receive the treatment regularly. Six cases (14.2%) didn't come to the 3rd month control. Twenty-three cases (54.7%) receiving the treatments regularly were evaluated at the 3rd month of the vitamin D treatment. The mean age of 23 cases was 13.4 ± 2.06 years. Fifteen cases (65.2%) were girls and 8 cases (34.8%) were boys. The changes in anthropometric measures and laboratory findings between before the treatment and the 3rd month of the treatment are given in Table IV. There were significant reductions in weight SDS, BMI SDS, hip circumference, total cholesterol, LDL, HbA1c, AST, interleukin 6 and PTH at the 3rd month of

the treatment. There was no significant change in levels of calcium, phosphorus and alkaline phosphatase, while a significant decrease was detected in PTH levels (p=0.016). Vitamin D level which had been 7.58 ± 2.7 ng/ml before the treatment increased to 23.49 ± 9.7 ng/ml after the treatment (p<0.0001). Vitamin D levels had reached up to sufficient levels in 22 of 23 cases (95.6%) at the 3rd month of the treatment. A comparison of the pre- and post-vitamin D treatmentshowed that HbA1c and AST levels had decreased significantly (p=0.049 and p=0.031, respectively), while no significant difference was observed in fasting glucose, fasting insulin, HOMA-IR, C-peptide and ALT levels. No significant difference was observed in levels of systolic-diastolic blood pressure, trigliseride, HDL cholesterol and hs-CRP between before and after the treatment. Total cholesterol, LDL cholesterol and IL-6 levels had decreased with the vitamin D treatment. While 5 of 23 cases (21.7%) had MS before the treatment, only 3 cases (13%) had MS after the treatment. There was no statistically significant difference in term of MS status after the treatment in comparison to before the treatment (p=0.36). The effect of change in BMI SDS on variables that changed significantly at the 3rd month of vitamin D treatment were evaluated by correlation analysis and it was found that a decrease in BMI SDS in the study group had no significant effect on changes in the total cholesterol, LDL, AST, HbA1c, interleukin-6 and parathormone levels (Table V).

Discussion

The mean serum 25(OH)D vitamin level of 10-18 year old obese adolescents in our study were 13.6 ± 7.8 ng/ml and 56.2% of obese cases had vitamin D deficiency. It has been revealed in many study that serum 25(OH) D vitamin levels of obese people are lower than the normal population and vitamin D deficiency is seen more frequently in obese people than the normal population.[3-5,21,22] In our study, cases with vitamin D deficiency were given 2000 IU/day vitamin D treatment for 3 months and their vitamin D levels increased significantly. Vitamin D levels had reached up to sufficient levels with the treatment in 95.6% of the vitamin D deficient cases. None of the cases had hypercalcemia during the treatment period.

A study by Javed et al.[8] evaluated 47 obese adolescents aged 12-18 years, in which 24 cases had taken 400 IU/day and 23 cases had taken 2000 IU/day vitamin D treatment for 3 months, results revealed that significant increase in vitamin D level was only seen with the 2000 IU/day vitamin D treatment group for 3 months.

Although obesity is associated with insulin resistance/hyperinsulinemia, attempts to diagnose insulin resistance by measuring plasma insulin concentration has no merit because it has no diagnostic value.[23] Therefore, there is no well-defined cut-off point differentiating normal from abnormal and no universally accepted, clinically useful, numeric

Table V. The Effect of Change in BMI SDS on Variables That Significantly Changed at the 3rd month of Vitamin D Treatment Evaluated By Correlation Analysis.

Variables	Effect of change in BMI SDS	
	r	p
Diastolic pressure	-0.042	0.801
LDL cholesterol	0.016	0.457
Total cholesterol	0.066	0.765
Parathormon	-0.103	0.639
AST	0.112	0.610
HbA1c	0.271	0.212
Interleukin 6	-0.06	0.784

BMI SDS: body mass index standart deviation score, HDL: high density lipoprotein, HbA1c: hemoglobin A1c, AST: aspartate aminotransferase

expression that defines insulin resistance.[24] Importantly, fasting insulin concentrations are similar in youths who are obese with normal glucose tolerance vs impaired glucose tolerance.[25] Because of these limitations, measuring plasma insulin concentrations remains a research tool with no clinical value for evaluation of obesity.[26] In our study, we used fasting insulin concentration and HOMA-IR for research purposes. The mean HOMA-IR value of obese adolescences in our study was 5.09 ± 2.87. The mean HOMA-IR value of obese adolescences was found to be 4.8 ± 4.7 by Javed et al.[8] while Belenchia et al.[27] revealed that value as 5.5 ± 2.7. In the Bogulasa Heart Study which was carried out between years 1988-1994 as a screening program in the USA, revealed that 4% of healthy children and 30% of obese children had IR.[28] In 2005 a study carried out in the UK revealed that 1/3 of obese children and young population had IR.[29] In our study 77% of 96 obese adolescences had IR. IR ratios for girls and boys were 77% and 66.2%, respectively. There was no statistically significant difference between vitamin D deficient and sufficient groups in term of the mean HOMA-IR value. Three months of 2000 IU/day vitamin D treatment had made no change in HOMA-IR. Like our study, Javed et al.[8] revealed that no significant change was observed in IR with 3 months of 2000 IU/day vitamin D treatment in 12-18 years aged vitamin D deficient obese adolescents. Nader et al.[30] revealed that there was no significant change in the parameters of glucose, insulin and HOMA-IR with 3 months of 2000 IU/day vitamin D treatment in 44 obese adolescents aged 12-18 years . Belenchia et al.[27] treated 21 obese cases aged 9-19 years of age whose mean 25(OH)D level was 19.2 ± 6.3 with 4000 IU/day vitamin D and evaluated them at the 3rd and 6th month. There was no change in BMI at the end of 6 month duration. There was a significant decrease in HOMA-IR value at the 6th month control, although no significant change was observed at the 3rd month control. In the same study a significant decrease in levels of fasting insulin and fasting glucose was also observed. This situation was thought to be related to anti-inflammatory and peripheral/hepatic glucose uptake acceleratory effects of vitamin D. In our study there was no difference between vitamin D deficient and sufficient groups in term of fasting insulin concentration and HOMA-IR and we observed no change in fasting insulin concentration and HOMA-IR with 3 months of 2000 IU/day vitamin D treatment. Therefore, we thought that vitamin D treatment in obese cases had no significant effect on fasting insulin and HOMA-IR in the short term. Studies examining vitamin D levels in association with childhood obesity usually do not consider the effect of insulin on vitamin D-binding protein and do not calculate the unbound, bioavailable vitamin D. Miraglia del Giudice et al.[31] showed total 25(OH)D levels lower in obese children compared to non-obese children. Bioavailable 25(OH)D levels were not different among the two groups. Insulin resistant children showed higher bioavailable levels of 25(OH) D compared to non-insulin resistant children and an inverse correlation between insulin resistance and vitamin D-binding protein was found. In our study, we were not able to evaluate bioavailable 25(OH)D levels. In order to clarify the relationship between obesity-insulin resistance-25(OH)D, it would be much more effective to measure the bioavailable 25(OH)D levels.

The frequency of MS has also increased as well as the frequency of obesity in children and adolescents. Cizmecioglu et al.[32] found that 20% of 131 obese cases aged between 2-18 years had MS. Atabek et al.[33] revealed the MS ratio as 20% in a 7-11 year old group and 27.2% in a 12-18 year old group. Bereket et al.[34] revealed that in 10-19 year aged adolescents MS prevalence according to IDF criteria was 2.3% in healthy children and 28% in obese children. In our study MS frequency in obese children was found as 25 % in accordance with the literature. In our study, MS frequencies of vitamin D deficient and sufficient groups were similar and there was no change in MS frequency by vitamin D treatment. Our study showed that a 2000 IU/day 3 month vitamin D treatment had no effect on MS as well as fasting insulin and HOMA-IR in vitamin D deficient group.

Roth et al.[35] evaluated the relation between vitamin D deficiency and IR in 125 obese and 31 non-obese children between the age of 6-16 years. They revealed that there was a reverse relation between HOMA-IR/HbA1c

and vitamin D level independent from BMI. It's claimed that the reason of the reverse relation between vitamin D and HbA1c levels might be the poor glycemic control which results from beta cell dysfunction secondary to inflammatory cytokines released from adipose tissue. This opinion was supported by the result of our study that with the Vitamin D treatment there was a significant decrease in HbA1c level although there was no change in levels of fasting plasma glucose, fasting insulin and C-peptide.

In our study, there was no significant difference between vitamin D deficient and sufficient groups in terms of systolic and diastolic blood pressure and lipid profile. However in the 3rd month of the vitamin D treatment there were significant decreases in levels of LDL and total cholesterol. There are few studies about effects of vitamin D treatment on hyperlipidemia and blood pressure in children. Javed et al.[8] evaluated the efficiencies of 400 IU/day and 2000 IU/day vitamin D treatments for 3 months in 47 obese adolescents aged 12-18 years, it was revealed that no change was observed in lipid profile by both treatment regimens. Similarly in another study, Nader et al.[30] revealed that there was no change in the lipid profile of 44 obese adolescents aged 12-18 years after a 3 month treatment of 2000 IU/day vitamin D. Pilz et al.[36] revealed that using 2800 IU/day vitamin D, no significant change had been observed in blood pressure and lipid profile of 200 adults who had arterial hypertension and vitamin D deficiency. Similarly it was also shown in the DAYLIGHT Study, in which effects of vitamin D were evaluated in 532 adult participants who had risk for hypertension, that vitamin D had no anti-hypertensive effect.[37] Unlike these studies in our study we observed decreases in levels of total cholesterol and LDL by the vitamin D treatment in obese adolescents.

Vascular structure and functions might be affected by the low level chronic inflammation results from cytokines released from increased adipose tissue in obesity. It has been shown in many studies that hs-CRP level by itself is an indicator for arterial stiffness index.[12] Proinflammatory cytokines like TNF-a, IL-6 and RBP-4 which increase during obesity contribute to vascular dysfunction.[11] In our study at the 3rd month of vitamin D treatment IL-6 level was decreased while hs-CRP level had no change. As far as we know there has been no study evaluating the relation between vitamin D treatment and pro-inflammatory cytokine levels in children. A study by Rodriguez-Rodriguez et al.[38] conducted with 137 obese children aged 9-12 years showed that IL-6 and hs-CRP levels were higher in vitamin D deficient children. In our study, however, levels of IL-6 and hs-CRP were found to be similar in vitamin D deficient and sufficient groups. In the study by Beilfuss et al.[39] they evaluated the relation of 40000 and 20000 IU/week vitamin D treatment to pro-inflammatory cytokine and IR in 332 adult obese people it was revealed that in both groups there was an increase in IL-6 levels and decrease in hs-CRP levels and no change in IR was observed. The decreased level of pro-inflammatory cytokine IL-6 by the treatment in our study is thought to be linked to the anti-inflammatory effect of vitamin D.

It was observed that the BMI SDS in the treatment group decreased. However, since our study was not double-blind randomized, it is not appropriate to directly relate this reduction of BMI SDS to vitamin D therapy. Confounding factors such as the treatment group being more motivated, and paying more attention to BMI status could have been effective in this reduction.

Our study has some limitations such as a short monitoring period, low number of participitants and the lack of a control group.

In conclusion, vitamin D deficiency in obese adolescents was found as 56.2% in our study and 96.5% of vitamin D deficient cases had reached sufficient vitamin D levels at the 3rd month of 2000 IU/day vitamin D treatment. At the end of the 3 months' total cholesterol and LDL cholesterol from cardiovascular risk parameters had decreased and the level of IL-6 had reduced, while insulin and HOMA-IR had no significant change. Although these parameters may have been effected by various factors, in this study we believe Vitamin D treatment to be the main reason that lies behind the changes. Larger scaled prospective randomized studies with longer follow-up periods are needed to obtain more informed outcomes on this topic.

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