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Serum Levels of Vitamin D, Retinol and Zinc in Relation to overweight among Toddlers: Findings from a National Study in Iran

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Abstract

Background: Some studies have examined the association between micronutrient deficiencies and overweight in children, but data in this regard are conflicting. This study was done to investigate the association between serum levels of vitamin D, A and zinc with overweight in a large sample of Iranian toddlers.

Methods: A total of 4261 toddlers, aged 15-23 months, who had an Iranian birth certificate and attended primary health care, were included in the current cross-sectional study. Weight and height were measured by experts based on standard protocols and body mass index (BMI) was calculated. Based on WHO criteria, overweight was defined as BMI-z-score of ≥1SD. Serum levels of $25(OH)D_3$, retinol and zinc were measured for each toddler. Binary logistic regression was applied to assess the association of $25(OH)D_3$, retinol and zinc levels with overweight.

Results: Mean age of study participants was 19.2 ± 8.4 months. After controlling for potential confounders, children in the highest quartile of serum $25(OH)D_3$ levels had lower odds of overweight compared with those in the lowest quartile (OR: 0.79, 95% CI: 0.63–0.99). Furthermore, a marginally significant inverse association was found between serum levels of $25(OH)D_3$ and overweight among urban toddlers (OR: 0.75, 95%CI: 0.56–1.00). Such a relationship was not seen for rural children. No other significant association was seen between serum levels of retinol and zinc and overweight either before or after controlling for covariates.

Conclusion: In conclusion, we found a significant inverse association between serum levels of vitamin D and overweight among toddlers. Further studies, particularly of prospective nature, are required to confirm our findings.

Keywords: BMI, Cholecalciferol, Micronutrient deficiency, Overweight, Retinol

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Introduction

Overweight and obese children are a growing health problem in developed and developing countries.[1] National studies in Iran have estimated that overweight and obesity is prevalent among 10.1% and 4.79% of Iranian children, respectively.[2] Overweight in Childhood is associated with greater odds of hypertension, type 2 diabetes, psychological disorders and also bone fracture in adulthood.[3-10]

Overweight is multifactorial and the interaction of genetic and environmental factors, including diet, is involved in its etiology. Among dietary factors, excess intake of energy and macronutrients is associated with greater risk of overweight.[11,12] However, the association between micronutrient deficiencies and overweight is unclear, particularly in children. It has been indicated that micronutrient deficiencies in children is associated with weak physical and mental development and more susceptibility to infection.[13,14] Furthermore, it increases risk of chronic disorders including cardiovascular diseases and other non-communicable diseases in adult life.[15,16] In parallel to the

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increasing prevalence of overweight, prevalence of undernutrition and micronutrient deficiencies is also growing in children under 5 years of age.[17] Therefore, there might be a link between malnutrition or micronutrient deficiency and overweight in children.

Among micronutrient deficiencies, vitamin D, A and zinc deficiencies are highly prevalent than others.[18,19] Some studies have examined the association between micronutrient deficiencies and overweight in children, but their findings are conflicting. Some of these studies indicated a significant positive association between micronutrient deficiency and overweight,[20-22] while others failed to find any significant relationship or reported inverse association.[23] Moreover, most previous publications have considered single nutrient deficiency rather than multiple deficiencies.[22,23] Assessing the association of multiple nutrient deficiencies with overweight provides more reliable information than evaluation of single nutrient deficiency. In addition, earlier studies on the association between micronutrient deficiencies and overweight among children had low sample size. Furthermore, earlier studies have been mostly done in non-Asian nations, and scarce data are available from the Middle East, particularly Iran. In this region, a high prevalence of micronutrient deficiencies among children has been reported.[24] Given the high prevalence of micronutrient deficiencies and overweight among Iranian children, the current study aimed to assess the association between serum levels of retinol, 25(OH)D_3 and zinc with overweight in a nationally representative sample of Iranian children aged 15-23 months.

Materials and Methods

Study Population

The present cross-sectional study was performed in framework of the National Integrated Micronutrient Survey 2 (NIMS-2) that was done in spring of 2012 in a large population of Iranian households living in urban and rural areas. NIMS-2 was performed by the School of Nutritional Sciences and Dietetics (SNSD), Tehran University of Medical Sciences (TUMS), Tehran, Iran, in collaboration with the Nutrition Department, Ministry of Health and Medical Education (MOHME), Tehran, Iran, with a close collaboration with the School of Public Health, TUMS, Tehran, Iran. Detail information on sampling and data gathering methods were published previously.[25] Briefly, in NIMS-2, all provinces of Iran were divided into 11 zones and then each zone was divided into 80 clusters. Totally, 880 clusters (including 530 urban and 350 rural) were selected proportionate to size around Iran. In each cluster, 5 individuals were randomly included. Therefore, 4,400 persons with age range of 15 to 23 months were assessed in NIMS-2. The reason for selection of this age group was that Iranian toddlers with this age range get one or two vaccines. During this time, blood sampling and data gathering were done. This age group can be considered as representative of toddlers aged between 12 and 36 months. In the current study, we excluded 139 toddlers due to incomplete information and therefore, 4261 children remained in the current analysis. All toddlers had Iranian birth certificates and attended health centers to get primary health care (urban and rural health units and rural health houses). Those who attended due to an illness (like common cold) were not included in the whole study. A signed informed written consent form was provided by parents of children. The Bioethics Committee of Tehran University of Medical Sciences, Tehran, Iran approved the study.

Assessment of Exposure

Assessment of micronutrient status was done biochemically. To do this, a 5 cc non-fasting blood sample was taken from all toddlers. Blood samples were stored in a temperature of minus 4 to 8°C. To examine vitamin D status, serum levels of cholecalciferol (25 (OH)D_3) was measured using electro-chemiluminescence immunoassay (ECLIA) on a Roche Elecsys system (Roche Diagnosis Elecsys). In the current study, those with a serum 25(OH)D_3 concentrations of <10 ng/mL were considered as vitamin D deficient and those with serum level of 10-30 ng/mL were defined as insufficient.[26] Vitamin A status was examined by measuring serum retinol concentrations using high performance liquid chromatography (HPLC, model: YL9100) with column temperature of 37 ° C and flow of 1 ml/min. Deficiency and severe deficiency of vitamin A were determined as serum retinol concentrations of <20 and <10 µg/dL, respectively.[27] Serum zinc concentrations were measured by Atomic Absorption (device model: Younglin ASS 8020). We defined zinc deficiency as serum zinc concentrations of <70 µg/dL.[28]

Anthropometric Measurements

Toddlers' weight and length were measured by an experienced expert or a trained technician at health centers of urban areas and health houses of rural areas. Weight was measured using a calibrated baby scale (used routinely in health centers) with minimum clothing and without shoes to the nearest 100 g. Length was measured on the supine position without shoes, using the UNICEF height-board (provided by UNICEF) to the nearest 0.5 cm. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters square. To calculate BMI-for-age z-score for each toddler, we used this formula: (calculated BMI - median BMI of the reference population) / standard deviation (SD) value of the reference population. The values of the reference population were obtained from WHO Child Growth Standards.[29] Based on WHO criteria, overweight was defined as BMI-for-age z-score of ≥1 SD.[30] Mid-upper arm circumference (MUAC) was measured by a standard procedure.[31]

Assessment of Other Variables

A self-administered questionnaire was used to collect information on age (month), family size (≥4/<4 members), first-rank birth (yes/no), birth interval with pre-child (<3/≥3 years), birth weight (kg), history of diseases (including diarrhea, respiratory infection, fever, epistaxia and fauvism) and supplement use (including vitamin A, D, zinc and iron

supplements).

Statistical Methods

We used the independent sample *t* test to examine significant differences between boys and girls as well as between toddlers living in rural and urban areas in terms of continuous variables. Distribution of participants in terms of categorical variables was examined by Chi-square test. Participants were categorized based on quartiles of serum 25(OH)D_3, retinol and zinc concentrations. One way analysis of variance (ANOVA) and Chi-square test were used to assess differences in terms of continuous and categorical variables, respectively, across quartiles of serum 25(OH)D3 levels. To assess the association of serum 25(OH)D_3, retinol and zinc levels with overweight, binary logistic regression was used in crude and adjusted models. In the adjusted model, age (continuous), family size (≥4/<4 members), first-rank birth (yes/no), birth interval with pre-child (<3/≥3 years or more), birth weight, history of diseases (diarrhea, respiratory infection, fever, epistaxia and fauvism), supplement use (including vitamin A, D, zinc and iron supplements) as well as serum 25(OH)D_3, retinol and zinc concentrations (where appropriate) were controlled. In all analyses, the first quartile of micronutrient levels was considered as the reference category. To compute the overall trend of odds ratios across increasing categories of micronutrient, we considered the quartiles of micronutrients levels as ordinal variables in the logistic regression models. All statistical analyses were done for the whole population as well as separately based on gender (boy/girl), area (urban/rural) and supplement use (yes/no) by SPSS software (version 21.0; SPSS Inc, Chicago IL). P values were considered significant at <0.05.

Results

Mean age of study participants was 19.2 ± 8.4 months and 46.8% were female. Prevalence of overweight was 19.7% (20.1% in boys and 19.2% in girls) among study participants.

Demographic characteristics of the total population, boys and girls as well as urban and rural toddlers, are indicated in Table 1. Boys were more likely to have higher birth weight, current weight, height, MUAC and serum 25(OH)D_3 concentrations than girls. In contrast, girls had higher serum levels of zinc than boys. In terms of geographical region, toddlers living in urban areas were more likely to be first-rank birth, have higher weight, height, MUAC, serum levels of zinc and retinol than those who lived in rural areas. Compared with those living in urban areas, rural toddlers had larger family size, higher serum 25(OH)D_3 concentrations and birth weight. Also, urban toddlers had higher interval with pre-child than rural ones.

Demographic characteristics of toddlers across quartiles of serum 25(OH)D_3 concentrations are presented in Table 2. Toddlers in the top quartile of 25(OH)D3 levels were more likely to be boy, rural, of small family size, use micronutrients supplements frequently, have greater interval with pre-child, higher serum zinc and retinol concentrations, and lower weight and height compared with those in the bottom quartile.

Multivariate adjusted odds ratios and 95% CIs for overweight across quartiles of serum 25(OH)D_3 concentrations are shown in Table 3. No significant association was found between serum 25(OH)D_3 concentrations and overweight in the crude model; however, after controlling for potential confounders, toddlers in the highest quartile of 25(OH)D_3 levels were 21% less likely to be overweight than those in the lowest quartile (OR: 0.79, 95% CI: 0.63–0.99). Stratified analysis based on residence revealed a marginally significant inverse association between serum levels of 25(OH)D_3 and overweight in urban toddlers (OR: 0.75, 95% CI: 0.56–1.00) but not in toddlers living in rural areas. Gender-stratified analysis showed no significant

Table 1. Demographic Characteristics of Toddlers Based on Gender and Geographical Region

Variables	Whole	Gender			Region		
		Boys	Girls	*P*[a]	Urban	Rural	*P*[a]
Age (mon)	19.2 ± 8.4	19.2 ± 8.6	19.2 ± 8.1	0.95	19.2 ± 10.1	19.4 ± 3.6	0.44
Family size (>4) (%)	28.3	28.5	28.8	0.87	24.2	36.8	<0.001
First-rank birth (%)	45.8	46.8	45.8	0.48	48.6	42.3	<0.001
Age interval with pre-child (>3 y) (%)	89.3	89.5	89	0.57	90.7	86.7	<0.001
Birth weight (kg)	3.4 ± 1.4	3.5 ± 1.4	3.4 ± 1.4	0.005	3.4 ± 1.2	3.6 ± 1.7	<0.001
Current weight (kg)	10.7 ± 1.6	11.0 ± 1.6	10.4 ± 1.6	<0.001	10.8 ± 1.6	10.7 ± 1.6	0.008
Height (cm)	81.9 ± 5.0	82.5 ± 5.0	81.2 ± 5.0	<0.001	82.1 ± 4.9	81.4 ± 5.2	<0.001
MUAC (cm)	15.0 ± 1.4	15.2 ± 1.4	14.9 ± 1.4	<0.001	15.1 ± 1.4	14.9 ± 1.4	<0.001
Zinc (μg/dL)	88.6 ± 21.9	87.9 ± 21.5	89.3 ± 22.2	0.03	89.4 ± 21.8	87.0 ± 21.9	<0.001
Retinol (μg/dL)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.64	0.3 ± 0.13	0.3 ± 0.1	0.02
25(OH)D_3 (ng/mL)	31.1 ± 15.6	31.9 ± 15.6	30.2 ± 15.4	<0.001	30.4 ± 15.8	32.4 ± 15.0	<0.001
Disease history[b] (%)	37.0	39.0	35.0	0.03	37.1	37.1	0.99
Supplement use[c] (%)	40.0	40.6	40.7	0.96	39.9	40.8	0.53

Abbreviation: MUAC, mid-upper arm circumference.
Data are presented as mean ± standard deviation (SD) or percent.
[a] Obtained from independent sample *t* test.
[b] Including diarrhea, respiratory infection, fever, epistaxia and fauvism.
[c] Including vitamin A, D, zinc and iron supplements.

Table 2. Demographic Characteristics of Toddlers Across Quartiles of Serum Levels of Vitamin D

Variables	Q1	Q2	Q3	Q4	*P*-trend[a]
Age	19.0 ± 2.8	19.5 ± 8.6	19.5 ± 9.9	19.0 ± 10.5	0.29
Sex (female) (%)	52.5	47.7	41.8	44.8	<0.001
Region (urban) (%)	72.3	62.7	60.9	62.2	<0.001
Family size (>4) (%)	28.8	31.1	29.1	25.5	0.03
First-rank birth (%)	44.9	44.2	46.8	49.4	0.06
Age interval with pre-child (>3 y) (%)	89.1	87.3	89.5	91.7	0.01
Birth weight (kg)	3.4 ± 1.3	3.5 ± 1.4	3.5 ± 1.5	3.4 ± 1.5	0.60
Current weight (kg)	10.9 ± 1.7	10.8 ± 1.5	10.8 ± 1.6	10.5 ± 1.7	<0.001
Height (cm)	82.1 ± 5.1	82.2 ± 5.0	82.0 ± 4.9	81.3 ± 5.0	<0.001
MUAC (cm)	15.1 ± 1.4	15.0 ± 1.4	15.0 ± 1.3	15.0 ± 1.5	0.10
Zinc (μg/dL)	86.7 ± 21.5	89.2 ± 22.9	88.7 ± 21.9	90.0 ± 21.1	0.004
Retinol (μg/dL)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.001
Disease history[b] (%)	37.4	38.1	38.2	37.6	0.98
Supplement use[c] (%)	30.8	34.6	43.2	53.5	<0.001

Abbreviation: MUAC, mid-upper arm circumference.
Data are presented as mean ± standard error (SE) or percent.
[a]Obtained from binary logistic regression.
[b]Including diarrhea, respiratory infection, fever, epistaxia and fauvism.
[c]Including vitamin A, D, zinc and iron supplements.

Table 3. Multivariate Adjusted Odds Ratio and 95% CI for Overweight Across Quartiles of Vitamin D Levels among Toddlers Aged 15-23 Months

	Q1	Q2	Q3	Q4	*P*-trend
Whole					
Crude	1	0.94 (0.76–1.16)	0.98 (0.80–1.21)	0.84 (0.68–1.04)	0.19
Adjusted model[a]	1	0.92 (0.74–1.14)	0.92 (0.74–1.14)	0.79 (0.63–0.99)	0.05
Boy					
Crude	1	0.98 (0.74–1.30)	1.02 (0.77–1.36)	0.85 (0.63–1.13)	0.35
Adjusted model	1	0.98 (0.73–1.32)	0.97 (0.72–1.31)	0.79 (0.58–1.07)	0.15
Girl					
Crude	1	0.81 (0.59–1.10)	0.86 (0.63–1.17)	0.78 (0.57–1.06)	0.16
Adjusted model	1	0.78 (0.57–1.08)	0.80 (0.58–1.11)	0.74 (0.53–1.03)	0.09
Urban					
Crude	1	0.97 (0.74–1.26)	1.01 (0.77–1.31)	0.82 (0.62–1.07)	0.21
Adjusted model	1	0.95 (0.72–1.25)	0.96 (0.72–1.26)	0.75 (0.56–1.00)	0.07
Rural					
Crude	1	0.75 (0.53–1.05)	0.86 (0.62–1.21)	0.82 (0.58–1.15)	0.41
Adjusted model		0.76 (0.53–1.09)	0.86 (0.60–1.22)	0.83 (0.58–1.19)	0.45
Using supplement[b]					
Crude	1	1.00 (0.72–1.38)	0.83 (0.60–1.16)	0.82 (0.59–1.15)	0.17
Adjusted model	1	1.01 (0.72–1.42)	0.86 (0.61–1.22)	0.84 (0.59–1.19)	0.19
Not-using supplement					
Crude	1	0.84 (0.64–1.11)	0.88 (0.67–1.16)	0.91 (0.69–1.19)	0.18
Adjusted model	1	0.82 (0.61–1.08)	0.84 (0.63–1.11)	0.84 (0.63–1.11)	0.11

Data are presented as OR (95 % CI)
[a]Adjusted for age, family size, first-rank birth, age difference with pre-child, birth weight, history of diseases (diarrhea, respiratory infection, fever, epistaxia and fauvism), supplement use (was not included in stratified analysis based on supplement use) as well as serum level of vitamin A and zinc.
[b]Vitamin A, D, zinc and iron supplements were considered.

association between serum $25(OH)D_3$ concentrations and odds of overweight either in boys or girls. When we divided toddlers by supplement use, such non-significant association was also seen either before or after controlling for confounders.

Multivariable-adjusted odds ratios and 95% CI for overweight across quartiles of serum retinol and zinc concentrations are presented in Tables 4 and 5. Neither in crude nor in adjusted model, we did not find any significant association between serum levels of retinol and zinc, and odds of overweight. Such non-significant association was also observed when the analyses were stratified based on gender, residence and supplement use.

Discussion

In the current study, we found an inverse association between serum levels of $25(OH)D_3$ and overweight among Iranian toddlers aged 15–23 months. This significant

Table 4. Multivariate Adjusted Odds Ratio and 95% CI for the Association between Retinol Levels and Overweight among Toddlers Aged 15–23 Months

	Q1	Q2	Q3	Q4	*P* trend
Whole					
Crude	1	1.02(0.83–1.26)	0.90 (0.72–1.12)	0.81 (0.65–1.01)	0.03
Adjusted model[a]	1	1.06 (0.86–1.32)	0.93 (0.74–1.17)	0.85 (0.67–1.07)	0.94
Boy					
Crude	1	0.98 (0.74–1.30)	0.86 (0.64–1.15)	0.77 (0.57–1.03)	0.05
Adjusted model	1	1.03 (0.77–1.38)	0.93 (0.68–1.25)	0.82(0.611–1.12)	0.17
Girl					
Crude	1	1.07 (0.79–1.47)	0.95 (0.70–1.30)	0.86(0.62–1.18)	0.28
Adjusted model	1	1.11 (0.80–1.52)	0.94(0.68–1.30)	0.87 (0.63–1.21)	0.30
Urban					
Crude	1	1.13 (0.86–1.48)	1.08 (0.83–1.41)	0.89 (0.68–1.17)	0.41
Adjusted model	1	1.10 (0.83–1.45)	1.05 (0.80–1.39)	0.88 (0.66–1.17)	0.37
Rural					
Crude	1	0.93 (0.66–1.31)	0.77 (0.54–1.10)	0.77 (0.54–1.08)	0.08
Adjusted model	1	0.95 (0.67–1.35)	0.83 (0.58–1.19)	0.83 (0.58–1.18)	0.23
Using supplement[b]					
Crude	1	0.94 (0.67–1.31)	1.15 (0.83–1.58)	0.85 (0.60–1.21)	0.37
Adjusted model	1	0.95 (0.67–1.35)	1.12 (0.80-1.57)	0.84 (0.59–1.21)	0.30
Not-using supplement					
Crude	1	1.30 (0.98–1.72)	1.35 (1.02–1.80)	1.21 (0.91–1.61)	0.09
Adjusted model	1	1.22 (0.91–1.63)	1.33 (0.99–1.78)	1.21 (0.91–1.62)	0.07

Data are presented as OR (95 % CI).
[a]Adjusted for age, family size, first-rank birth, age difference with pre-child, birth weight, history of diseases (diarrhea, respiratory infection, fever, epistaxia and fauvism), supplement use (was not included in stratified analysis based on supplement use) as well as serum level of vitamin D and zinc.
[b]Vitamin A, D, zinc and iron supplements were considered.

Table 5. Multivariate Adjusted Odds Ratio and 95% CI for the Association between Zinc Levels and Overweight among Toddlers Aged 15–23 Months

	Q1	Q2	Q3	Q4	*P*-trend
Whole					
Zinc					
Crude	1	1.15 (0.93–1.43)	1.09 (0.88–1.34)	1.09 (0.88–1.35)	0.54
Adjusted model[a]	1	1.22 (0.97–1.52)	1.15 (0.92–1.44)	1.18 (0.94–1.48)	0.22
Boy					
Zinc					
Crude	1	1.37 (1.03–1.83)	1.27 (0.95–1.71)	1.09 (0.81–1.48)	0.68
Adjusted model	1	1.45 (1.07–1.97)	1.32 (0.97–1.80)	1.23 (0.90–1.68)	0.31
Girl					
Zinc					
Crude	1	0.87 (0.64–1.19)	0.90 (0.66–1.23)	1.01 (0.75–1.37)	0.86
Adjusted model	1	0.93 (0.67–1.29)	0.98 (0.70–1.35)	1.04 (0.75–1.43)	0.74
Urban					
Zinc					
Crude	1	1.11 (0.85–1.46)	1.12 (0.86–1.47)	1.10 (0.84–1.45)	0.48
Adjusted model	1	1.15 (0.86–1.53)	1.17 (0.88–1.56)	1.14 (0.85–1.53)	0.35
Rural					
Zinc					
Crude	1	1.07 (0.76–1.51)	1.05 (0.74–1.49)	1.00 (0.70–1.42)	0.97
Adjusted model	1	1.06 (0.74–1.51)	1.08 (0.75–1.55)	1.06 (0.73–1.52)	0.74
Using supplement[b]					
Crude	1	1.23 (0.88–1.72)	1.16 (0.83–1.62)	1.00 (0.71–1.42)	0.34
Adjusted model	1	1.36 (0.95–1.93)	1.25 (0.88–1.78)	1.12 (0.78–1.61)	0.46
Not-using supplement					
Crude	1	1.10 (0.83–1.45)	1.04 (0.79–1.37)	1.15 (0.87–1.51)	0.64
Adjusted model	1	1.11 (0.83–1.49)	1.07 (0.80–1.42)	1.19 (0.89–1.59)	0.73

Data are presented as OR (95 % CI)
[a] Adjusted for age, family size, first-rank birth, age difference with pre-child, birth weight, history of diseases (diarrhea, respiratory infection, fever, epistaxia and fauvism), supplement use (was not included in stratified analysis based on supplement use) as well as serum level of vitamin D and A.
[b] Vitamin A, D, zinc and iron supplements were considered.

association was seen when potential confounders were taken into account. Furthermore, in the fully adjusted model, a marginally significant inverse association was found between serum levels of 25(OH)D_3 and overweight in urban toddlers. No other significant association was seen between serum levels of retinol, zinc and overweight. To the best of our knowledge, this study is the first study to examine the association of serum 25(OH)D_3, retinol and Zinc levels with overweight among a representative large sample of toddlers in Iran.

Considering the high prevalence of overweight, finding the contributing factors to its prevalence is of great importance.[32-34] We found an inverse association between serum 25(OH)D_3 levels and odds of overweight among toddlers. Although a large number of studies have examined the association of serum 25(OH)D_3 levels with overweight and obese in children and adults, we are aware of no study to examine such association in toddlers. In a cohort study on healthy adults, an inverse association was found between serum levels of 1, 25-dihydroxy vitamin D and risk of obesity.[35] In a meta-analysis, higher BMI was associated with lower 25(OH)D_3 concentrations, while any effects of low 25(OH)D_3 on increasing BMI were small. Therefore, investigators suggested that reduction of BMI is a solution for decreasing prevalence of vitamin D deficiency.[36] In a cross-sectional study, overweight and obese children had significantly greater odds of vitamin D deficiency compared with those with normal weight.[37] Such findings were also reported in some previous studies.[38-41] In contrast, findings from three clinical trials indicated that vitamin D supplementation had no significant effect on weight or BMI.[42-44] The effect of vitamin D on weight or BMI in children might be different because they are in their growth period and their requirements are different compared with adults. Therefore, further studies, in particular clinical trials, are needed to assess the effect of vitamin D supplementation on weight or BMI among children.

The reasons for the observed region disparity in the association between serum levels of vitamin D and overweight are unclear. It might be explained by different educational and economic status and dietary intakes of individuals living in urban and rural regions. It seems that pregnant and lactating women living in urban areas have better dietary intakes compared with those that lived in rural areas.[45] However, sun exposure in rural areas may be more than urban areas.[46]

Several hypotheses have been presented for the inverse association between vitamin D levels and overweight. Low serum levels of vitamin D are associated with low levels of calcium.[47] The inverse association between serum levels of calcium and overweight had been previously reported.[48] Intracellular calcium increases the lipolytic activity of human adipocytes.[49] It has been shown that vitamin D might protectively influence the risk of overweight by adjusting the catabolic and anabolic activity of adipocytes. When vitamin D levels rise, activity of enzymes catabolizing adipose tissue increases.[50] In contrast, low levels of vitamin D are associated with stimulation of anabolic-enzyme activity of adipose tissue.[50-52] In addition, vitamin D can inhibit the expression of a key abiogenesis regulator, peroxisome proliferator-activator-gama that is associated with fat accumulation.[49,50] Some experimental studies have demonstrated that vitamin D inhibits the autogenesis of adipocytes and induces the adipocyte apoptosis.[49,53]

Enrollment of a representative sample of Iranian toddlers and assessing a very specific age group can be considered as strengths of the current study; however, several limitations should be mentioned for interpretation of our findings. Based on the cross-sectional design of our study, we cannot confer a causal link between vitamin D status and overweight in toddlers. Therefore, our findings should be confirmed by prospective studies. Seasonal variations of vitamin D levels might affect our findings. However, individuals recruited in the current study were examined in more than one season, but we cannot exclude the confounding effects of seasonal variation. Although several confounders were controlled to obtain an independent association between serum levels of micronutrient and overweight, further adjustment for other confounders including age of starting food, sun exposure, season of blood drawing, intake of breast milk or formula, education and economic status of households, skin color of children and maternal dietary intakes during pregnancy might be needed. Therefore, these adjustments should be considered in future studies.

In conclusion, a significant inverse association was found between serum levels of vitamin D and overweight in Iranian toddlers. Furthermore, such inverse association was also seen among urban toddlers. However, this association was marginally significant. No other significant association was seen between serum levels of retinol, zinc and overweight. Further studies, particularly of prospective nature, are required to confirm our findings.

Authors' Contribution

YS and OS contributed to data preparation and manuscript drafting. AD and FS assisted in designing the study. MJ, ADJ and KM conceptualized and oversaw this study, MP and ZA provided guidance on the analysis, and provided substantial contributions to the editing of the paper. RH and AY took primary responsibility for drafting this manuscript and provided guidance on the analysis. HP and AE conducted the analysis and provided substantial contributions to the editing of the paper. All authors approved the final version of manuscript.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Bioethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

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