Open Access Macedonian Journal of Medical Sciences. 2014 Dec 15; 2(4):562-566.
http://dx.doi.org/10.3889/oamjms.2014.101
Basic Science

25-Hydroxy Vitamin D, Adiponectin Levels and Cardiometabolic Risk Factors in a Sample of Obese Children

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Citation: Hassan NE, El-Masry SA, El Banna RA, Abu Shady MM, Al-Tohamy M, Mouhamed Ali M, Abd El-Moniem MM, Anwar M. 25-Hydroxy Vitamin D, Adiponectin Levels and Cardiometabolic Risk Factors in a Sample of Obese Children. OA Maced J Med Sci. 2014 Dec 15; 2(4):562-566. http://dx.doi.org/10.3889/oamjms.2014.101

Key words: 25-Hydroxy vitamin D; adiponectin; lipid profile; blood pressure; children.

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Received: 18-Aug-2014; **Revised:** 24-Sep-2014; **Accepted:** 02-Oct-2014; **Online first:** 26-Oct-2014

Competing Interests: The authors have declared that no competing interests exist.

Abstract

BACKGROUND: Association between vitamin D, adiponectin and obesity is a matter of debate, as they play important role in linking obesity with different cardio metabolic risk factors.

AIM: Evaluation of association between metabolic risk factors with both adiponectin and 25-Hydroxy vitamin D [25(OH) D] levels and that between adiponectin and [25(OH) D] among obese Egyptian children.

SUBJECTS AND METHODS: This case-control cross sectional study consisted of 65 obese and 30 healthy children, aged 8-11 years. 25(OH) D, serum adiponectin, total cholesterol, triglycerides, high-density lipoprotein-cholesterol and low-density lipoprotein-cholesterol were measured.

RESULTS: The mean 25(OH)D and adiponectin levels in the obese were lower than that in control group (P<0.000). 25(OH)D were inversely correlated with body mass index, triglyceride, total cholesterol and LDL-cholesterol. While adiponectin level were inversely correlated with systolic and diastolic blood pressure, and positively correlated with high-density lipoprotein-cholesterol. However, there is no relation between 25(OH) D and adiponectin levels among obese children and total sample.

CONCLUSION: Inspite of strong association between vitamin D and adiponectin levels with metabolic risk factors and obesity, there is no relation between 25(OH)D and adiponectin levels. In obese children, There are significant negative correlations between 25(OH)D with lipid profile, and between adiponectin levels with blood pressure.

Introduction

Obesity is one of the most serious public health problems in the 21st century [1]. Childhood obesity has been known to be associated with a range of health problems, which may last until adult life and cause premature morbidity and mortality [2]. Evidence is accumulating to suggest that there is a potential link between obesity and Vitamin D deficiency among global populations [3]. Vitamin D (1,25-dihydroxycholecalciferol; Calcitriol) is essential for normal growth and development. Recent studies have suggested that vitamin D deficiency is associated with cardiometabolic risk factors, autoimmune diseases, insulin resistance, abnormal lipid profile, and high blood pressure (BP) in adults [4]. However, few studies have evaluated the relationship between 25(OH)D concentrations and the cardiometabolic risk factors in children and adolescents, first was Reis et al., [5] from USA and the last research from Italy was Walker et al., [6].

Adiponectin is also emerging as an important mediator of risk for cardiovascular disease [7]. Clinically, hypoadiponectinemia is associated with both obesity and cardiovascular disease, whereas high serum adiponectin levels correlate with improved cardiovascular function and atherosclerosis [8]. Many researchers have observed decreasing adiponectin levels in association with increased obesity in children [9, 10].

Kardas and his colleagues [11], suggested that the interaction between vitamin D and adiponectin levels may be an indicator of cardiometabolic risk factors such as atherosclerosis. Studies regarding the association between vitamin D and adiponectin levels with cardiometabolic risk factors in obese and healthy children are few.

So, the purpose of this research is to evaluate the association between cardio metabolic risk factors with both adiponectin and 25(OH)D levels and that between adiponectin and 25(OH)D levels among obese Egyptian children.

Subjects and Methods

This case-control cross sectional study consisted of 65 obese children (32 males and 33 females), and 30 control non-obese ones (15 males and 15 females) with age range 8-11 years. They were derived from a cross-sectional survey, conducted on 2083 child (874 boys and 1209 girls), from 2 primary public schools, randomly selected, situated in Giza governorate, Egypt. Pupils were excluded if they had a prior major illness, including type 1 or 2 diabetes, received medications or had a condition known to influence their physical growth.

Prior to enrollment, permission to perform the study was granted by the Ministry of Education, and the directors of the schools included in the research. Parents were fully informed about the purpose and methods of the study and signed a written consent. The children provided their verbal assent. Approval to conduct this survey was granted by the "Ethical Committee" of the “National Research Centre”. All information and blood samples were collected by a well-trained staff during morning visits to the schools.

The study sample was divided into 2 groups (obese and non-obese) by body mass index (BMI) (kg/m^2), which was percentile-specific for sex and age of Egyptian children and adolescents [12]. Healthy, age- and sex-matched children were selected as control. The BMI of the obese children was greater than the 95^{th} percentile for age and sex, while the BMI of the control children ranged between 15^{th} and 85^{th} percentiles based on the Egyptian Growth Reference Charts [12].

Anthropometric assessment

Each child underwent a complete physical examination. Then, anthropometric measurements including weight in kilograms (kg) and height in centimeters (cm); for each child; were performed by the same trained doctor using standard devices and following the recommendations of the International Biological program [13]. Height was measured to the nearest 0.1 cm on a Holtain portable anthropometer, and weight was determined to the nearest 0.01 kg using Seca Scale Balance, with the subject wearing minimal clothing and no shoes. BMI; as weight (kg) divided by height $(m)^2$; was calculated. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice using a mercury sphygmomanometer, after the subject rested for at least 20 min.

Biological Parameters

Early morning forearm venous blood samples (5 ml) were obtained from each child; before breakfast; for biochemical screening tests after 12-hours overnight fasting. Professional staff performed venipuncture. The blood samples were left to clot; sera were separated by centrifugation for 10 minutes at 5000 rpm then stored at -80ºC until assays. Plasma 25(OH) D levels were measured by high-pressure liquid chromatography (HPLC) using Clin- Rep kits (IRIS Technologies International GmbH, Cursdorf, Germany). Serum adiponectin levels were measured using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits, provided by AviBion Orgenium Laboratories, Finland. Serum concentrations of total cholesterol (TC: normal range, 160–200 mg/dL), triglycerides (TG: normal range, 40–140 mg/dL) and high-density lipoprotein-cholesterol (HDL-C: normal range, 35–80 mg/dL) were measured using commercially available kits provided by STANBIO Laboratory Inc. (1261 North Main Street Boerne Texas 78006 USA).

LDL-C (normal range, 100–130 mg/dL) was calculated according to an equation developed by Friedewald et al. [14], as follows:

LDL-C= Total cholesterol –Triglycerides/5+ HDL-C.

Statistical Analysis

Data analysis was performed using SPSS version 16.0 (SPSS. Inc., Chicago, IL, USA). The results were expressed as mean ± SD. The Kolmogorov-Smirnov test was used to determine the normality of the data, and revealed that it was not normally distributed. Differences between groups were analyzed using the Mann-Whitney test. Spearman’ s correlation coefficients were used to assess relationships between independent variables. The level of significance was set at a probability of less than 5% ($p < 0.05$).

Results

The mean 25(OH)D levels in the total sample, males and females were 33.02 ± 11.97, 35.71 ± 10.61, and 30.89 ± 12.64 ng/mL respectively. There was significant sex difference in 25(OH)D levels in the

control group only; where males had the higher values; while insignificant sex difference was recorded between the obese group and total sample. However, insignificant sex difference in adiponectin level was recorded between the total sample and both groups (Table 1). Moreover, insignificant sex difference in the blood pressure and lipid profile was found in obese and control groups. So, the analysis was completed without sex differentiation.

Table 1: Sex differences in 25-hydroxy Vitamin D and adiponectin levels in obese and control.

	Total		Males		Females		Z-value	P
	Mean	±SD	Mean	±SD	Mean	±SD		
Obese (N= 65):								
25-hydroxy Vit D (ng/ml)	29.93	±10.34	32.00	±7.69	27.92	±12.17	- 1.734	0.083
Adiponectin (µg/ml)	1.76	±0.43	1.80	±0.45	1.71	±0.409	- 0.881	0.378
Control (N=30):								
25-hydroxy Vit D (ng/ml)	39.73	±12.67	47.60	±10.15	35.80	±12.13	- 2.313	0.019*
Adiponectin (µg/ml)	4.01	± 0.83	3.99	± 0.83	4.02	± 0.87	-0.145	0.902
Total (N=95):								
25-hydroxy Vit D (ng/ml)	33.02	±11.97	35.71	±10.61	30.89	±12.64	- 1.923	0.055
Adiponectin (µg/ml)	2.47	±1.20	2.51	±1.19	2.43	±1.23	- 0.529	0.597

*, P<0.05 = Significant differences.

Comparisons between the descriptive characteristics of obese and control groups of the study sample (clinical and biological parameters) are shown in Table 2. The mean ages of the study population, obese group, and control group were 9.99 ± 1.14, 10.1 ± 1.18 and 10.27 ± 0.74 years, respectively with insignificant differences. While SBP, DBP, TG and TC were significantly higher in the obese group than those in control group ($P < 0.01$), whereas LDL-C level showed insignificant difference between the two groups. On the other side, HDL-C, 25(OH)D, adiponectin were significantly higher in the control group than those in obese group ($P < 0.01$ for all variables) (Table 2).

The present study revealed insignificant correlation between 25(OH) D and adiponectin levels for the total sample and obese group (Table 3, 4). However, for total sample and obese children; 25(OH)D levels showed a negative significant correlation with age, BMI, TG, TC and LDL-C levels and insignificant correlation with blood pressure - both systolic and diastolic, and HDL-C. While, adiponectin level showed negative significant correlations with blood pressure - both systolic and diastolic, and significant positive one with HDL-C, for both total sample and obese group.

At the same time, adiponectin level showed a negative significant correlation with BMI, TG and TC and insignificant ones for the obese group. This means that there is significant negative relation between adiponectin level with BMI and lipid profile up to certain level. When obesity occurs (BMI>95th percentile), these relations disappear; i.e. the changes in adiponectin level becomes not related to the changes in either BMI or lipid profile (TG, TC and LDL) (Table 3, 4).

Discussion

Recent studies suggested association of obesity with cardiovascular diseases and both 25 hydroxy vitamin D and adiponectin levels in children and adolescents [11]. Obesity is usually correlated with the higher prevalence of hypovitaminosis or the lower circulating 25(OH) D level in both pediatric as well as adult populations [15]. Low vitamin D stores as determined by 25-hydroxy vitamin D [25(OH) D] levels; which is the most accurate way to measure how much vitamin D is in the body (normal, insufficiency or deficiency); are associated with increased risk for cardiovascular and mortality, but the mechanisms for this are unclear [16].

The prevalence of 25(OH)D deficiency was higher in the obese group (29.9 ng/mL) than in the control group (39.7 ng/mL) in the present study. In India, Shivaprakash and Joseph [17] also reported deficiency of 25(OH) D levels among 35obese children (17.49 ng/mL). This indicated the high prevalence of vitamin D insufficiency/ deficiency in the countries that receive abundant sunshine like India and Egypt. Even among American children, Johnson et al., [18], found that the mean level for 25(OH)D was 28.8 ng/mL in 197 obese children. Cheng et al., [19], also stated that both subcutaneous and visceral adiposity are associated with low 25(OH) D concentrations. The inverse relationship between obesity and serum 25(OH) D concentrations may have several explanations, including deposition of vitamin D in body fat compartments, reduced release of vitamin D into systemic circulation and low exposure to sun light [20]. Until now, data regarding the role of vitamin D in obesity are inconclusive [21].

Table 2: Comparisons of BMI, blood pressure and lipid profile between obese and control.

	Total (N=95)		Obese (N= 65)		Control (N=30)		Z-value	P
	Mean	±SD	Mean	±SD	Mean	±SD		
Age (years)	9.99	±1.14	10.10	±1.18	10.27	±0.74	- 0.658	0.510
BMI (Kg/m$^{2)}$	24.28	±5.95	27.90	±2.928	16.44	±1.67	7.809	0.000**
SBP (mmHg)	110.88	±12.66	112.83	±14.618	106.67	±4.61	2.880	0.004*
DBP (mmHg)	70.58	±11.53	72.69	±13.178	66.00	±4.03	4.207	0.000**
TG (mg/dL))	119.98	±41.29	133.22	±34.398	91.28	±40.88	5.362	0.000**
TC (mg/dL)	172.25	±57.45	188.72	±60.53	136.57	±26.24	4.205	0.000**
HDL-c (mg/dL)	33.94	±17.43	21.27	± 4.88	39.87	±8.04	-5.162	0.000**
LDL-c (mg/dL)	126.47	±53.15	133.91	± 4.95	122.99	±56.58	0.933	0.351
25-hydroxy Vit D (ng/ml)	33.02	±11.97	29.93	±10.34	39.73	±12.66	- 3.372	0.001**
Adiponectin (µg/ml)	2.47	±1.20	1.76	± 0.43	4.01	± 0.83	-7.812	0.000**

*, P<0.05 = Significant differences; **, P<0.001 = highly significant differences.

Statistically significant inverse association of serum 25(OH) D with BMI and age without sex difference was detected in this study. This result has been found to be similar to Kardas and his colleagues, [11] in Turkey. Abu Shady et al., [24], also found inverse association of serum 25(OH) D with BMI among 215 Egyptian school children, but with insignificant relation with age and sex. On contrary, Shivaprakash and, Joseph [17] in India and Johnson et al., [18] in America found insignificant relation between 25(OH) D levels and either BMI, age or sex.

Table 3: Spearman's correlations between 25-hydroxy Vit D, adiponectin and cardiometabolic risk factors among total sample.

	25-hydroxy Vit D)		Adiponectin	
	R	p	r	p
Age (years)	- 0.296	0.004*	- 0.110	0.290
BMI (Kg/m$^{2)}$	- 0.260	0.036	- 0.587	0.000**
SBP (mmHg)	0.187	0.136	- 0.434	0.000**
DBP (mmHg)	0.006	0.963	- 0.500	0.000**
TG (mg/dL))	- 0.288	0.020*	- 0.389	0.000**
TC (mg/dL)	- 0.413	0.001**	- 0.325	0.001**
HDL-c (mg/dL)	0.234	0.062	0.267	0.009*
LDL-c (mg/dL)	- 0.265	0.034*	0.048	0.643
25-hydroxy Vit D (ng/ml)			- 0.202	0.107
Adiponectin (µg/ml)	- 0.202	0.107		

*, P<0.05 = Significant differences; **, P<0.001 = highly significant differences.

Current results showed that adiponectin was lower in obese children than control. Epidemiological evidence supports a protective role for adiponectin in cardiovascular disease. Low serum adiponectin is also predictive of a future coronary artery ischemic event and the development of hypertension and the levels of the adipokine correlate with left ventricular hypertrophy. High serum adiponectin is associated with better recovery of cardiac function post-injury [22].

Table 4: Correlation between 25-hydroxy Vit D, adiponectin and cardiometabolic risk factors among obese children.

	25-hydroxy Vit D		Adiponectin	
	R	p	r	p
Age (years)	- 0.410	0.001**	0.237	0.058
BMI (Kg/m$^{2)}$	- 0.260	0.036*	0.185	0.140
SBP (mmHg)	0.187	0.136	- 0.346	0.005*
DBP (mmHg)	0.006	0.963	- 0.313	0.011
TG (mg/dL))	- 0.288	0.020*	0.154	0.220
TC (mg/dL)	- 0.413	0.001**	0.067	0.594
HDL-c (mg/dL)	0.234	0.062	0.359	0.004*
LDL-c (mg/dL)	- 0.265	0.034*	-0.091	0.475
25-hydroxy Vit D (ng/ml)			- 0.202	0.107
Adiponectin (µg/ml)	- 0.202	0.107		

*, P<0.05 = Significant differences; **, P<0.01 = highly significant differences.

Moreover, the present study revealed that lipid profile and blood pressure were significantly higher in obese children as compared to control. This profile predisposes obese children to cardiovascular disease later in life as mentioned before in different studies [17, 23].

Several reports are available about the association between 25(OH) D and plasma lipids. In this study, statistically significant inverse correlation was identified between 25(OH) D levels and the lipid profile except HDL level .This result agree with that of Abu Shady et al., [24]. On the other hand, Dolinsky et al., [25], found insignificant association between 25(OH)D level and triglycerides, total cholesterol, LDL or HDL. While Kardas and his colleagues, [11] found that 25(OH) D levels were positively correlated with HDL, LDL, total cholesterol, triglyceride (TG), systolic blood pressure (SBP), and diastolic blood pressure (DBP). In theory, 25(OH) D could affect lipid profile directly as it is essential for maintaining adequate levels of apolipoprotein. In addition, the indirect effects of 25(OH) D on lipid profile could be through parathyroid hormones (PTH) or calcium balance [26].

Current results provided the finding that 25(OH)D is not associated with adiponectin. This result agreed with Walker et al., [6] in Italy. In contrast, some recent studies have suggested that 25(OH)D may play a role in the regulation of adiponectin [11, 15]. Adiponectin was identified and confirmed to be significantly decreased in 25(OH)D deficient obese pediatric [16]. Kardas et al., 2013 [11] in Turkey reported that 25(OH)D was positively correlated with adiponectin.

Adiponectin level significantly correlates negatively with blood pressure parameters, both systolic and diastolic, among both total and obese children in the present study. This finding confirms the results of Brambilla et al., [27] in their study on 186 obese Italian children ranging in age from 5–18 years. On the other hand, Li et al., [28] stated that, this association remains inconclusive because obese subjects may have different mechanisms to regulate blood pressure. They also, recommended that larger studies are needed to examine the relationship between adiponectin and mechanisms of blood pressure regulation.

The present results showed that adiponectin was associated with significant positive correlation with HDL-C. In agreement with these findings, Tamang et al., [29] reported that serum adiponectin correlates positively with HDL-C levels in obese subjects. They suggested that the possible mechanisms may partially be explained with the activated receptor, which affects the genes, associated with HDL metabolism. Adiponectin stimulated ligand activates in liver and skeletal muscles, which results in the increased synthesis of HDL-C.

This study has some important differences as compared with those previously discussed; that there is significant negative relation between adiponectin level and BMI up to certain level of BMI. When obesity occurs (BMI>95th percentile), these relations disappear; i.e. the changes in adiponectin level becomes not related to the changes in BMI. This means that in obese children, the increase in their BMI is not associated with further decrease in adiponectin level.

In conclusion, early detection of these risk factors (especially 25(OH)D and adiponectin) in

children can prevent the increasing risk of cardiovascular diseases.

Acknowledgments

We would like to acknowledge our institute "National Research Centre' Egypt" - without its fund this study could not be done. We would also like to acknowledge everybody participated in this study, the children who were the participants of this study, the technicians who helped in the laboratory analysis and the doctors who participated in collection of the data. Without their help, this study couldn't have been completed.

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