Electronic Journal of General Medicine
2020, 17(6), em255
e-ISSN: 2516-3507
https://www.ejgm.co.uk/

Original Article

MODESTUM
OPEN ACCESS

Alkaline Phosphatase is not Associated with Insulin Resistance: A Retrospective Cross-Sectional Study

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Citation: Gürler EB, Çeçen S. Alkaline Phosphatase is not Associated with Insulin Resistance: A Retrospective Cross-Sectional Study. Electron J Gen Med. 2020;17(6):em255. https://doi.org/10.29333/ejgm/8323

ARTICLE INFO

Received: 11 Apr. 2020

Accepted: 26 May 2020

ABSTRACT

Background: Many epidemiologic studies suggested that vitamin D deficiency is associated with obesity but the relationship between obesity and alkaline phosphatase (ALP) is controversial. In this study association between insulin resistance and alkaline phosphatase is investigated.

Methods: Of the 124 women aged (35,942±9,714) years with (39,47±7,66) body mass index (BMI) and with vitamin D deficiency is included. The relationship among insulin, glucose, parathormone (PTH), calcium (Ca), alkaline phosphatase (ALP) were evaulated with correlation, linear regression and ANOVA (with post hoc TUKEY). Statistical analysis performed with Eviews v.11 and Graphpad Prism v.8.1

Results: There was no significant correlation among Vitamin D, ALP and PTH in non-insulin resistance patients. A weak correlation was found among Vitamin D, ALP and Parathormone in insulin resistance group. There was no significant elevation between ALP levels in patients with or without insulin resistance.

Conclusion: Elevation in ALP levels is not associated with insulin resistance. It might be a response to elevated parathormone levels. Further investigations in large populations are required to understand the relationship between insulin resistance and ALP.

Keywords: alkaline phosphatase, insulin resistance, parathormone, vitamin D

INTRODUCTION

Vitamin D3 or cholecalciferol are two main forms of vitamin D and obtained by the action of UVB light on cholesterol in the skin (1). There is a small contribution from food and other supplements (2). Vitamin D3 and cholecalciferol are prohormones that are hydroxylated in the liver to 25OH and finally in the kidney into 1,25-dihydroxy vitamin D3 [1,25(OH)2D]. This active form binds to vitamin D receptors inside cells and forms gamma carboxy glutamic acid-containing protein or Ca^{+2}-binding protein which mediates the absorption of calcium from the gut (3). In normal physiological conditions, this process is regulated by parathyroid hormone (PTH) and calcium in circulation.

Alkaline phosphatase (ALP) was first described in 1907 (4) and investigated extensively. However, there is a limited knowledge about its physiological role. Many studies revealed that elevated level of ALP is associated with bone and liver diseases [5-8]. On the contrary, lower levels of ALP is also related with several disorders including; osteoporosis, malnutrition, magnesium deficiency and hypothyroidism (9-11).

Indeed, vitamin D is a fat-soluble vitamin, meaning that it can be stored in body fat (BF). Excessive BF can reduce 25(OH)D levels in the body, especially in obese individuals.

Limited studies have assessed the association between obesity, 25(OH)D levels, parathormone, and ALP in non-menopause obese women. This retrospective cross-sectional study aimed to understand the relationship among vitamin D, parathormone, and ALP status among Turkish obese women by analyzing data from the Marmara University Pendik Research and Education Hospital Sports Physiology Clinics.

MATERIALS AND METHODS

Study Population

Of the 124 women aged (36±10) years with (40±8) body mass index (BMI) and serum 25(OH)D levels of around 13 were (13.26±5.63) included in this study. Exclusion criteria was limited to patients that are taking vitamins, Ca^{+2}, undergoing therapies related to bone metabolism. Pregnant women, patients suffering from hypo/hyperthyroidism, and dyslipidemia were also excluded from the study.

Measurement of Obesity and Insulin Resistance

Obesity status was measured based on BMI and homeostatic model assessment (HOMA). Bodyweight (BW), fat mass (FM), fat-free mass (FFM), fat percentage (% F), was measured in light clothing and without shoes on a BC-418 Segmental Body Composition Analyzer (Tanita, Tokyo, Japan).

Table 1. General Characteristics of Study Population (N=124)

	Age	BMI	25(OH)D	PTH	ALP	HOMA	F %	FM	FFM
Mean	35.942	39.471	13.266	53.359	77.235	4.445	44.131	46.0389	56.526
Median	38.000	37.500	11.900	49.100	75.420	3.550	43.600	44.200	55.300
Mode	18.000	30.3	10.300	**27.500**[a]	**59.000**[a]	**1.7**[a]	43.600	56.200	49.300
Sum	4456.89	4894.4	1645.01	6616.55	9577.26	551.2	5472.24	5708.82	7009.332

a. Multiple modes exist. The smallest value is shown

Table 2. Correlation among variables (Overall)

	N=124	BMI	25(OH)D	PTH	ALP	HOMA	% F	FM	FFM
BMI	Pearson Correlation	1	**-.384****	**.241****	**.185***	**.494****	**.825****	**.915****	**.764****
	Sig. (2-tailed)		.000	.007	.039	.000	.000	.000	.000
25(OH)D	Pearson Correlation	**-.384****	1	**-.358****	-.173	**-.250****	**-.310****	**-.390****	**-.353****
	Sig. (2-tailed)	.000		.000	.055	.005	.000	.000	.000
PTH	Pearson Correlation	**.241****	**-.358****	1	**.339****	.105	.129	.168	**.229***
	Sig. (2-tailed)	.007	.000		.000	.245	.153	.063	.010
ALP	Pearson Correlation	**.185***	-.173	**.339****	1	**.245****	.140	**.208***	**.183***
	Sig. (2-tailed)	.039	.055	.000		.006	.120	.021	.042
HOMA	Pearson Correlation	**.494****	**-.250****	.105	**.245****	1	**.326****	**.480****	**.511****
	Sig. (2-tailed)	.000	.005	.245	.006		.000	.000	.000
% F	Pearson Correlation	**.825****	**-.310****	.129	.140	**.326****	1	**.902****	**.491****
	Sig. (2-tailed)	.000	.000	.153	.120	.000		.000	.000
FM	Pearson Correlation	**.915****	**-.390****	.168	**.208***	**.480****	**.902****	1	**.773****
	Sig. (2-tailed)	.000	.000	.063	.021	.000	.000		.000
FFM	Pearson Correlation	**.764****	**-.353****	**.229***	**.183***	**.511****	**.491****	**.773****	1
	Sig. (2-tailed)	.000	.000	.010	.042	.000	.000	.000	

Height was measured to the nearest 1 mm by a stadiometer. BMI was calculated as [bodyweight (kg)/height $(m)^2$]. For adult women, we used the World Health Organization criteria for the Caucasian population, which defines obesity as a BMI ≥25 kg/m^2. HOMA of β-cell function and insulin resistance (IR) was calculated using the formula: FBI (µU/L) x FBG (nmol/L)/22.5.

Biochemical Analysis

Blood samples were obtained in the morning after a fast of at least 8 hours. Analysis of plasma concentrations of insulin, glucose, PTH, Ca, ALP was processed in the Pendik Education and Research Hospital Central Laboratory, İstanbul. Insulin and PTH were assessed on a Cobas®6000 (Roche Diagnostics, Mannheim, Germany), and on a Dimension Vista®1500 (Siemens Healthcare, Erlangen, Germany) using enzymatic colorimetric method. Ca, ALP, and glucose were assessed on AU®5900 (Beckman Coulter, USA) using photometric, kinetic, and enzymatic colour tests. Vitamin D was determined on Access®Unicel (Beckman Coulter, USA) using a chemiluminescent immunoassay (EIA).

Statistical Analysis

Pearson correlations were performed to explain the associations among variables. Variables that were significantly associated with the dependent variables on Pearson analyses were evaluated in the linear regression models. A linear regression analysis was used to summarize and study relationships between variables. Statistical analysis was performed using Eviews (v.11, USA). One-way ANOVA and post-hoc Tukey were also performed to investigate the relationship between PTH, ALP, and insulin resistance.

Statistical analysis was performed according to obesity (BMI>29), insulin resistance (HOMA<2.7 or HOMA>5.5), or parathormone level (15<PTH< 65 pg/ml or PTH>65 pg/ml).

RESULTS

General characteristics of participants are presented in **Table 1**. On average, participants had high body mass index (40±8 kg/m2). They were divided into three groups: 2.41 % overweight (Class I obesity), 51.6 % Class II obese, 48.4 % Class III. Average serum vitamin 25(OH)D levels were 13.26 ± 5.63 ng/mL and all subjects had vitamin D insufficiency. PTH levels were 53.35±20.89 pg/mL with 40% of them were at the maximum level (65 pg/ml). Average ALP level was 77.23±19.70 U/L. HOMA was 4.45±2.81 while 25 % of patients did not have insulin resistance. Association between Vitamin D, PTH, Ca, ALP, HOMA, F %, FM, FFM, and BMI are shown in **Table 2**. BMI was inversely correlated with Vitamin D insufficiency and significantly associated with PTH, ALP, HOMA, F %, FM, and FFM, while Vitamin D was not associated with ALP.

Association between vitamin D insufficiency and PTH and ALP was further analyzed by stratifying the insulin resistance group (**Tables 3** and **4**). There was no significant correlation among Vitamin D, ALP, and PTH in non-insulin resistance group (data not shown). On the contrary, there was a significant correlation among Vitamin D, ALP, and PTH (**Table 3**) in the insulin resistance group. However, this was not significant in the regression analysis (**Table 4**). No significant difference was observed between ALP levels in patients with or without insulin resistance, while a significant decrease was seen in Vitamin D levels ($p<0.01$) and an elevation in PTH levels ($p<0.05$) (**Figure 1**). Concomitant with PTH, there was a significant ($p<0.001$) increase in FM and FFM (**Figure 2**).

When the study group was classified according to PTH levels (15<PTH< 65 pg/ml or PTH>65 pg/ml) a significant increase in ALP levels ($p<0.05$) and a significant decrease in Vitamin D levels ($p<0.001$) (**Figure 3**) was observed.

Table 3. Correlation among variables (Insulin resistance +)

Correlations				
N=32		25(OH)D	ALP	PTH
25(OH)D	Pearson Correlation	1	**-.403***	**-.502****
	Sig. (2-tailed)		.022	.003
ALP	Pearson Correlation	**-.403***	1	**.392***
	Sig. (2-tailed)	.022		.026
PTH	Pearson Correlation	**-.502****	**.392***	1
	Sig. (2-tailed)	.003	.026	

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 4. Linear regression analysis among variables (Insulin resistance +)

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	**.403**[a]	.163	.135	4.683673757853097
a. Predictors: (Constant). ALP				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
2	**.502**[a]	.252	.227	4.425881320297497
a. Predictors: (Constant), PTH				

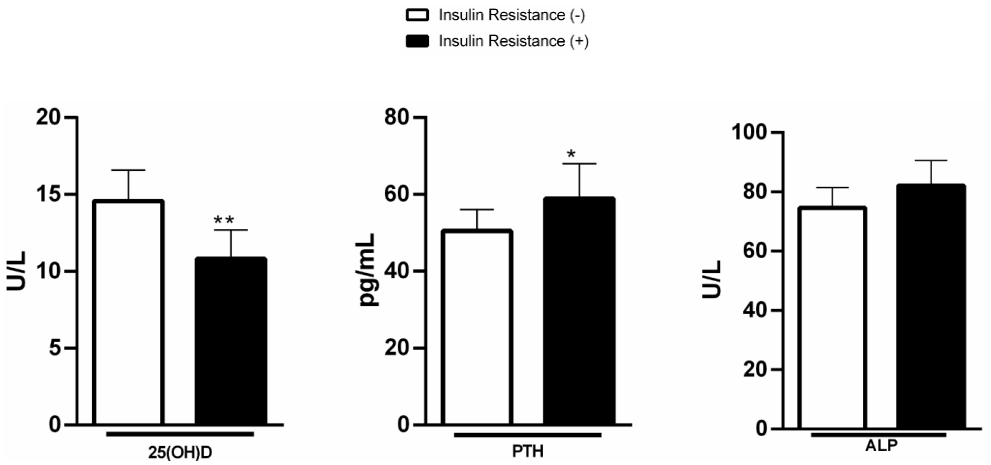

Figure 1. Variation between groups (Vitamin D, PTH, ALP). *p<0.05 and **p<0.01.

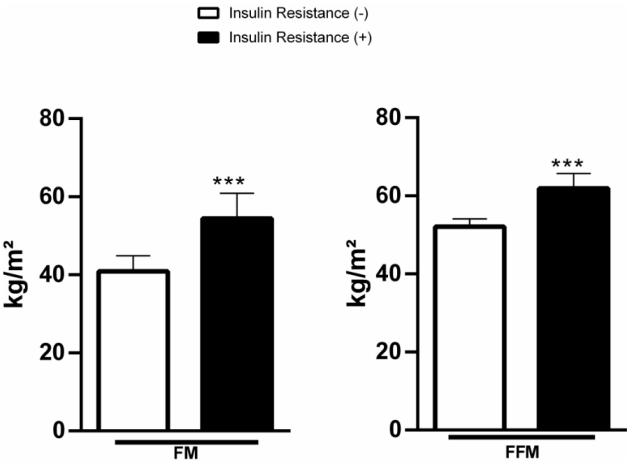

Figure 2. Variation between groups (FM, FFM). ***p<0.001

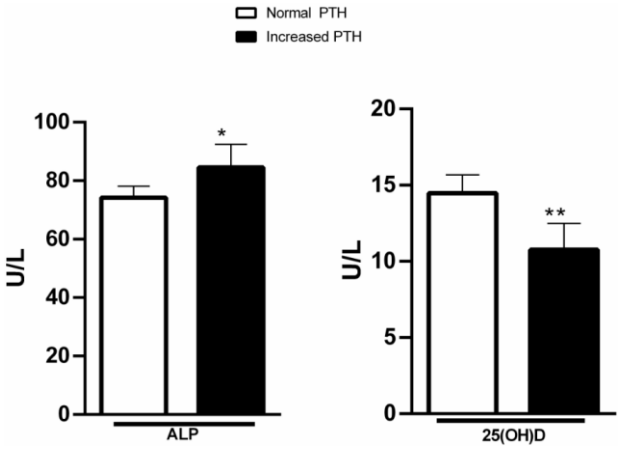

Figure 3. Variation between groups (ALP, 25OHD). *p<0.05 and **p<0.01

DISCUSSION

We conducted a cross-sectional study to investigate the association between vitamin D insufficiency and insulin, PTH, ALP, and obesity among Turkish adult women aged 18-51 years using integrated data from Pendik Education and Research Hospital Sports Physiology Clinics. Similar to the previous reports (12,13), our results revealed a significant inverse relation between Vitamin D and obesity. On the other hand, we determined that there was no association between serum vitamin D and ALP levels before and after adjustment for IR based on HOMA.

Prevalence of vitamin D deficiency is variable. A meta-analytic study revealed a correlation between obesity and vitamin D deficiency is stronger in the Asian population than in the European-American population. Furthermore, it is also related to skin color, clothing style, regional differences (14-16). A Turkish study showed that vitamin D levels were low in 66.6% of women of reproductive age in Istanbul (17) and it was 93% in the entire female population (18).

However, several studies have pointed out an inverse association between vitamin D deficiency and obesity, it is still unclear whether low vitamin D status is responsible for the development of obesity or whether obesity causes vitamin D deficiency. There is a meta-analytic study also showed a weak correlation between serum 25(OH) D levels and BMI in the adult population (19).

Additionally, previous experiments suggested that reduction in Vitamin D levels may lead to greater adiposity by stimulating parathormone levels and overflow of calcium into adipocytes, thereby increasing lipogenesis (20). Concomitant to these findings, we find a significant inverse association with Vitamin D and FM, FFM, and F %.

Several reports also showed a significant elevation in the serum ALP level in obese individuals (21-23). It is known that ALP is expressed in the adipose tissue (24). Controversially to previous reports, our results suggested that association among FM, FFM, F %, and ALP is not sufficient. Similar to our findings, a study revealed no direct relation of vitamin D deficiency with ALP (15), and another study also pointed out Vitamin D concentrations were not related to ALP in drug-naïve individuals without liver disease (25). Besides, there was also no significant cross-sectional association between 25(OH)D and bone turnover markers (26).

Overall, this study has several limitations. First of all, we could not exclude the possibility of residual confounding factors like sun exposure on 25(OH)D levels, bone diseases, non-alcoholic fatty liver disease. Second, we identified the association between Vitamin D, ALP, PTH, and insulin resistance. However, due to the nature of a cross-sectional study, we had problems in identifying causal relationships between exposure and outcome. Therefore, well-designed studies, more homogenous study populations, and clinical trials, are needed to confirm this causality.

CONCLUSION

In conclusion, prevalence of vitamin D insufficiency or deficiency among adult women is considerably high, and the serum 25(OH)D levels of these women are inversely associated with HOMA and BMI similar to previous reports. Parallel to vitamin D, PTH is associated with vitamin D insufficiency but ALP does not show a direct relationship with insulin resistance. Elevation in ALP levels may be a response to PTH. Further investigations in large populations are required to understand the relationship between insulin resistance and ALP.

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