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ASSOCIATION OF SERUM VITAMIN D AND OMENTIN-1 IN PATIENTS WITH CORONARY ARTERY DISEASE.

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ABSTRACT… Objectives: The aim of the present study was to investigate the association between serum vitamin D (calcediol, D2) with omentin-1 in patients with coronary artery disease (CAD). **Study Design:** Case Control Study/ Cross Sectional Study. **Setting:** Civil Hospital Karachi (CHK). **Period:** January 2016 to June 2016. **Material & Methods:** In this cross-sectional study, total of 250 cases of coronary artery disease were recruited randomly from the civil hospital, Karachi. Diagnosis based upon coronary angiography. Serum Vitamin D and omentin levels determined by using enzyme linked immunosorbent essay (ELISA) in Dr. Abdul Qadeer khan Institute of Biotechnology and Genetic Engineering (KIBGE). 120 apparently healthy controls were recruited. **Results:** we observed significant low levels of serum vitamin D and omentin-1in patients of coronary artery disease. Vitamin D (calcidiole, D2) deficiency (<30ng/L) was found in 82 % (n= 205) of CAD patients, moreover; 50.8% (n=127) patients were found with severe vitamin D deficiency (8.77± 3.87) placed in group III, 31.2% (n=78) patients were found with moderate deficiency (17.09 ±4 ng/mL) in group II, whereas only 18% (n=45) had optimal serum vitamin D levels (33.02 ±16.00 ng/ mL) placed in group I. Mean serum omentin-1 level was (578± 21.87, 409±32.09, 321± 23.01) in these subgroups respectively. Serum vitamin D (calcidiole, D2) level was associated positively with omentin-1 in CAD patients (P = 0.002) after adjustment for potential confounding variables; basal metabolic rate, waist circumference, blood pressure and lipid profile. **Conclusion:** Within the limits of the study, we concluded that low levels of vitamin D and omentin-1 are associated with prevalent coronary artery disease (CAD) and are independent of other cardiovascular risk factors. Further investigations are required in different ethnic groups and populations to confirm the findings.

Key words: Coronary Artery Disease, Omentin-1, Vitamin D.

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INTRODUCTION

Vitamin D (sun vitamin), is a fat soluble vitamin. It has two major types; ergo-calcidiol (vitamin D2), found in plants and chole-calciferol (vitamin D3), found in fish, oil or synthesized by the skin when exposed to sunlight from the precursor molecule 7-dehydrocholesterol.[1] There is hydroxylation of both forms in the liver to form 25-hydroxy-cholecalciferol, represent the vitamin D deposits in body also used to determine vitamin D sufficiency or deficiency, due to its longer plasma half-life.[2] In addition to fundamental share in calcium metabolism, bone health and mineral homeostasis, now the role of vitamin D in endocrine system has been established. Studies have shown its involvement in immune system and increasing or decreasing expression of certain cytokines.[3] Similarly, it is also involved in the development of athermanous plaque by enhancing the lipid uptake via monocytes/ macrophage system and their conversion into foam cells.[4] Several research studies have suggested the association of coronary artery disease (CAD) and vitamin D insufficiency via metabolic functions, insulin sensitivity, and endothelial dysfunctions.[5-6] The presence of the vitamin D receptors in adipose tissue and pre-adipocytes discloses a direct role for vitamin D in regulating adipokcytokine gene expression. Active form of vitamin D involve in inhibition of pro-inflammatory adipocytokines production whereas stimulates anti-inflammatory adipokines secretion from the adipose tissues through decrease expression of the nuclear

factor Kappa-B (NFκB). Concurrently, Vitamin D deficiency accelerates CAD progression through enhanced chronic inflammation by activation of protein KPNA4 which in turns stimulate the activation of inflammatory factor called nuclear factor kappa-B (NF-κB) These are the novel outcomes provide knowledge about beneficial preventive and pharmacological effects of vitamin D supplementation in CAD.[7-8]

Omentin-1 is a 34-kDa, anti-inflammatory, circulating adipocytokine, has been considered to have a significant role in endothelial dysfunction, atherosclerosis and myocardial remodeling.[9] Omentin-1 exhibit its anti-inflammatory role by hindering tissue necrosis alpha (TNF-alpha) factor that is a pro-inflammatory cytokine. It activates activated B cells in endothelial cells via nuclear factor kappa-light-chain. Omentin-1 also activates protein Kinase (5'AMP) that inhibit expression of vascular adhesion molecule E-selectin.[10]

OBJECTIVE

Literature has revealed the association of Low Vitamin D and omentin-1 levels with cardiovascular disease11-12 , however, the correlation of serum vitamin D concentrations with omentin-1 is far less been studied within CAD patients. So the objective of this study is to determine the association of vitamin D with serum omentin-1 concentrations and other cardio metabolic risk factors in CAD patients.

MATERIAL & METHODS

This case-control, cross sectional study was conducted in civil hospital Karachi (CHK) from January 2016 to June 2016, after obtaining approval from ethical board committee of Dr. Abdul Qadeer Khan Institute of Biotechnology and Engineering (KIBGE). There was recruitment of 250 (157 males, 93 female) patients of CAD. The diagnoses of CAD was made on the basis of electrocardiographic (significant Q waves >2 mm, ST depression >2mm and T-wave inversion in more than one ECG leads), history of chest pain for more than30 minutes, Positive Troponin I test (> 0.01ng/ml) moreover, the patients with more than 50% obstruction of one or more major coronary arteries declared by angiography, were considered the patients of CAD. The exclusion criteria were acute infections, malignancy, valvular heart disease, liver disease (ALT > 58 units/L), renal disorders (Creatinine > 1.5 mg/dl) and pregnancy. Subjects on anti-inflammatory drugs and vitamin D supplements were excluded from study. Age and sex matched 220 apparently healthy controls were included in this study. All candidates provided informed written consent prior the study.

The information about study variables including age, sex, exercise, socioeconomic status, consumption of junk food, smoking status, family history of heart disease, hypertension, diabetes mellitus, use of anti-hyperlipidemic, anti-diabetic, antihypertensive drugs; were collected through Performa, designed for the research.

Anthropometric parameters including body mass index (BMI), waist circumference (cm), height (feet), and weight (Kgs) were measured. The BMI was calculated by the formula (kg/m^2).

There was collection of 5ml venous blood from brachial artery of patients after overnight fasting between 8:00 am to 9:00 am in vacationers containing EDTA, then centrifuged for 5 min, plasma was separated and frozen at -80 C in sterile Eppendorf till the day of essay. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum omentin-1 concentrations (Bio Vender, USA).

The serum vitamin D concentration was measured by automatic direct electro-chemiluminescence immunoassay (Roche Diagnostics). The lower limit of measurement was 3.9mg/dl.

According to serum vitamin D status, participants were categorized in 3 groups:
Group (I); sufficient levels vitamin D ≥ 30ng/ml
Group (II); moderate deficiency of vitamin D=10-29 ng/mL
Group (III); serious deficiency of vitamin D = 0.9-9.0 ng/mL

STATISTICS

Statistical analysis of results was conducted with

SPSS version 16. (Chicago, USA). Unpaired t- test was the statistical method used for comparing quantitative variables among groups. All variables were presented in mean ± SD. Spearman's rank correlation was used to observed the relationship between serum vitamin D, omentin-1 and cardio-metabolic risk factors in CAD. Multivariable linear regression was used to analyze the relationship of vitamin D and omentin-1 with adjustment for other study parameters like; age, basal metabolic rate, waist circumference, systolic and diastolic blood pressures, lipid profile and blood sugar.

RESULTS

The average age of CAD patients was (53.29±5, 5.51±4.82, 54.98±5.74) respectively in three subgroups while that of the control group was (55.43± 4.90) years. Vitamin D deficiency (<30ng/ mL) was found in 82 % (n= 205) of CAD patients, however; 127 (50.8%) patients were found with severe vitamin D deficiency (8.77± 3.87) in group III, 78(31.2%) patients were found with vitamin D moderate deficiency (17.09 ±4 ng/mL) in group II, whereas only 45 (18%) had optimal serum vitamin D levels (33.02 ±16.00 ng/ mL) in group I. Mean serum omentin-1 level was (578± 21.87, 409±32.09, 321± 23.01; ng/ mL) in these groups respectively. In controls mean vitamin D level was found 31.08± 8.08 ng/ mL whereas, mean omentin-1 levels were 680± 43.09 ng/ mL. No significant differences were observed in three subgroups of cases of CAD with respect to age, body mass index (BMI), waist circumference, blood pressure, fasting blood glucose, total cholesterol, HDL-C, values. However, serum omentin-1, serum triglycerides and LDL-c values showed significant variance between the groups as well with controls. (Table-I).

Table-II is demonstrating Positive correlation between vitamin D levels and serum omentin-1 levels ($r = 0.654$; $p < 0.01$, $r = 0.718$; $p < 0.01$, $r = 0.698$; $p < 0.01$) in three groups, respectively. Negative correlations was found between serum vitamin D levels and total cholesterol (−0.053; −0.046; −0.039), LDL-C (−0.001; -0.012; -0.018), although, were not significant. However negative correlations between vitamin D and waste circumference ($r = -0.229$; **$r = -0.229$**; $r = -0.241$; $p < 0.01$**) and** TG ($r = -0.323$; $r = -0.317$; $r = 0.330$; $p < 0.01$) was found to be statistically significant.

Study Variables	CAD (n= 250)			Controls n=220	P-Value
	Group I (n=45)	Group II (n=78)	Group III (n=127)		
Age (year)	53.29±5	55.51±4.82	54.98±5.74	55.43± 4.90	0.528
BMI (kg/m^2)	31.38±3.37	32.41±3.28	30.77±2.95	29.03±5.67	0.182
WC (cm)	35.5± 4.4	34.09± 5.0	33.21± 5.76	37.12± 6.67	0.122
SBP (mmHg)	136.15±16.19	139.54±13.22	137.38±11.68	140.2± 10.0	0.131
DBP (mmHg)	81.31±9.25	84.27±7.24	78.19±6.13	88.09± 12.34	0.165
FBS (mg/dl)	116.29±21.57	109.43±16.72	123.42±8.56	102± 9.32	0.018
TC (mg/dL)	196.14±21.27	188.26±18.39	179.35±15.28	187± 11.0	0.011
TG (mg/dL)	121.91±17.22	113.15±13.36	124.27±11.15	99± 11.09	0.003*
HDL (mg/dL)	32.86±3.75	38.73±4.14	43.52±6.78	39.22±8.02	0.109
LDL (mg/dL)	119.31±14.61	128.86±11.54	123.14± 9.15	91.31±16.01	0.007*
Vit D (ng/mL)	33.02 ±16.00	17.09 ±4	8.77± 3.87	31.08± 8.08	0.001*
Omentin-1(ng/mL)	578± 21.87	409±32.09	321± 23.01	680± 43.09	0.008*

Table-I. Comparison of clinical, demographic and anthropometric characteristics of study population.

Variables	Patients with CAD (n = 250)			P value
	Group I r value	Group II r value	Group III r value	
BMI (kg/m²)	**0.152**	**0.235**	**0.133**	**0.026**
WC (cm)	– 0.229*	– 0.229*	– 0.241*	0.001 *
TC (mg/dL)	– 0.053	– 0.046	– 0.039	0.286
LDL-c (mg/dL)	–0.001	-0.012	-0.018	0.004*
HDL-c (mg/dL)	0.141	0.119	0.142	0.024
TG (mg/dL)	– 0.323**	– 0.317**	0.330**	0.006*
FBS(mg/dL)	0.109	0.100	0.110	0.072
SBP (mmHg)	0.058	0.061	0.080	0.081
DBP (mmHg)	0.066	0.036	0.060	0.035
Omentin-1 (ng/mL)	0.654**	0.718**	0.698*	0.001*

Table II: Correlations between serum vitamin d with omentin-1 and different cardio-metabolic risk factors in CAD patients

	Model 1	Model 2	Model 3	Model 4
Vit D ≤30ng/ml	3.45 (1.59-7.64)	3.98 (1.54-7.79)	3.90 (1.7-8.66)	3.23 (1.44-7.24)
P value	0.002*	0.002*	0.001*	0.003*

Table-III. Multiple linear regression analysis for the association between vitamin D (independent variable), omentin-1(dependent variable) in CAD patients.

Model 1 is unadjusted, 2=adjusted for anthropometric parameters, 3= adjusted for biochemical parameters, 4= adjusted for all studied parameters; $P < 0.01$ is significant. In multivariable regression analysis (Table-III), four models were made to confirm the association between Omentin-1 (dependent variable) and vitamin D (independent variable). Positive association was observed between omentin-1 and vitamin D levels in unadjusted model 1 (r= 3.45, CI; 1.59-7.64, $p = 0.002$). After controlling for biophysical parameters (BMI, WC, SBP and DBP) in Model 2 and biochemical parameters in model 3 strong correlation was found between omentin-1 and vitamin D (r= 3.98, CI; 1.54-7.79, $p = 0.002$) (r= 3.90, CI; 1.7-8.66, $p = 0.001$). After further controlling all studied parameters in model 4 significant correlation was still found (r= 3.23, CI; 1.44-7.24, $p = 0.003$).

DISCUSSION

In the present study we aimed to determine the association between vitamin D and serum omentin-1 levels in CAD patients. The outcomes of our study revealed that there is a strong relationship between serum vitamin D (calcidiol, D2) and omentin-1 levels. Low serum levels of calcidiol (D2) were found with decrease secretion of omentin-1 in CAD patients. These results agreed with the research study piloted by Dikker et al., who observed the calcidiol (D2) and omentin-1 levels in postmenopausal females. Increased omentin-1 levels were observed in women with normal vitamin D levels. A positive correlation between calcidiol (D2) levels and omentin-1 was found in all groups made according to vitamin D serum concentrations.[13] Another study conducted by zorlu et al; discovered the negative relationship between calcidiol (vitamin D2) and omentin-1serum levels in healthy female volunteers.[14] Fazelian et al. have compared omentin-1 levels and vitamin D, before- and after-treatment, in female patients of type 2 diabetes mellitus. He observed significantly raised omentin-1 levels with high levels of vitamin D.[15] Very few studies have been conducted to assess the relationship between vitamin D and omentin-1, however; literature has given the association between

vitamin D and other adipokines. Maggi S et al., observed increased serum leptin levels with vitamin D therapy in the type 2 diabetic patients.[16] Similarly, Gangloff et al; observed the positive correlation between vitamin D and leptin in young males with central obesity.[17] Mohammad SM et al. worked on another adipokine adiponectin in diabetic patients and found high vitamin D levels with high adiponectin values.[18] Some studies have shown the Positive correlation of vitamin D with interlukin-10 which is an anti-inflammatory cytokine while negative correlation with pro-inflammatory cytokine interleukin-6.[19-20-21] These studies have demonstrated the vital role of Vitamin D in adipocytokines production via adipocytes in different disease. Researches have been done to explain the mechanisms of action of vitamin D on adipokines secretion and it is It is suggested that vitamin D receptors are present on adipose cells and they might alter the expression of adipocytokine genes.[22]

Low serum vitamin D concentrations have been found to associate with abnormal lipid profile. Diabetes mellitus and cardiovascular diseases often accompanied by abnormal levels of TC, LDL-c, HDL-c and TG.[23] Ford et al. revealed negative correlation between serum vitamin D concentrations and Triglycerides in healthy males.[24] Moreover, wang et al. has given data about negative association of vitamin D with TC, LDL-c and TG and positive with HDL-c.[25] High LDL-c and TG, with low HDL-c have observed in our samples of CAD, we found statistically significant negative correlation between serum vitamin D TG and LDL-c in CAD patients.

It is important to note that in current study, although, we found positive and negative relationship between serum vitamin D and different anthropometric and biochemical parameters in CAD patients, but all the results were not significant. A possible elucidation for the non-significant results may be due to the lingering and impenetrable impacts of medicines commonly used by participants with CAD.

CAD remains the major reason of mortality and morbidity in Pakistan. The identification of the pathophysiological mechanism of inflammation and confounding risk factors is essential for preventive and treatment strategies of disease. On the basis of evolving facts about the association of vitamin D, adipokocytokines and inflammation in coronary artery disease, it is hypothesized that vitamin D may control the systemic inflammatory process through release of adipocytokines. Studies have reported that vitamin D inhibit pro-inflammatory cytokines secretion whereas increases the expression of anti-inflammatory cytokines. Based on the understanding of this association, the focus of researchers has been in the correction of vitamin D deficiency with the purpose of preventing diseases and improving the prognosis of established disease.

The strength of our study is that we are the first to report the association between serum vitamin D concentrations with omentin-1 with CAD patient. Secondly young to middle age patients were recruited to the risk of the confounding systemic disease.

As vitamin D and omentin-1 gene play an anti-inflammatory role, their deficiency might be a potential risk factor for development of CAD. However, further study is needed on large sample size to confirm the findings.

LIMITATION OF STUDY

- Small sample size:
- Both metabolites of vitamin D are not measured.
- Confounding factors such as socioeconomic status, physical activity and junk food are not mentioned.

CONCLUSION

In present study we conclude that patients of CAD had significant low level of serum vitamin D and omentin-1 as compared to controls. Secondly, a statistically significant positive correlation was found between serum vitamin D and omentin-1 levels.

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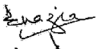
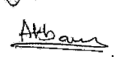
1	Shazia Nazar	Main idea / Write up, Statistics.	
2	Ambreen Qamar	Data / Sample collection.	
3	Akbar Mughal	Sample collection.	
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