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Research Article

Study of Possible relation between Vitamin-D status and subclinical Atherosclerosis

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Abstract

Background: Vitamin-D and PTH (parathyroid hormone) may influence cardiovascular risk through a shared association with atherosclerotic plaque formation and progression. However, both vitamin D and PTH have been inconsistently found to be associated with early signs of atherosclerosis, such as increased carotid intimal-medial wall thickness (IMT) which can be determined with B-mode ultrasound . ***Objectives:*** the study aimed to investigate possible effect of vitamin-D status on carotid intimal thickness, in relation to insulin like growth factor level. ***Results:*** About 31% of studied population had low vitamin-D level as well as 11% of them had high CIMT **.** Regarding relation of vitamin D and CIMT ,our study showed a statistically significant positive correlation between CIMT and 25(OH) vit D in the studied male group(r=.437, P=.001)but found a significant inverse correlation between the same two parameters in the studied female group(r=-.606,P=.000). ***Conclusion:*** vitamin D seemed to play important role in the pathophysiology of atherosclerosis , but it is still unclear at what stage(s) in the atherosclerotic disease process vitamin D may exert its effects.

Keywords: vitamin-D, intimal medial thickness , Insulin like Growth Factor -1.

Introduction

An early sign of atherosclerosis is hypertrophy of the arterial wall. Increased intimal-medial thickness (IMT) is a non-invasive marker of arterial wall alteration, which can be easily assessed in the carotid arteries by high-resolution B-mode ultrasound.(1).

Vitamin-D and PTH (parathyroid hormone) may influence cardiovascular risk through a shared association with atherosclerotic plaque formation and progression. However, both vitamin D and PTH have been inconsistently found to be associated with early signs of atherosclerosis, such as increased carotid intimal-medial wall thickness (IMT) (2,3)

There is accumulating evidence that vitamin-D may influence vascular function and the development or progression of atherosclerosis. As vitamin D receptors have a broad tissue distribution which includes vascular smooth muscle cells [4,5] and macrophages [6].

The interplay between vitamin-D and IGF-1 (insulin like growth factor-1) is complex and occurs at both endocrine and paracrine/autocrine levels. Vitamin-D has been shown to increase circulating IGF-1 and IGFBP-3(insulin like growth factor binding protein-3). The modulation of IGF-1 and IGFBP-3 concentrations by vitamin-D may impact recombinant human (rh) GH dosing for the treatment of Growth hormone deficiency .It might also underlie some of the extra-skeletal beneficial effects ascribed to vitamin D.(7)

Experimental evidence indicates that IGF-1 counteracts vascular aging and atherosclerosis, for which increased carotid artery intimal - medial

thickness (IMT) is a marker. Yet, IGF-1 concentrations have been inconsistently associated with carotid IMT in epidemiological studies (8).

Also, recent studies found vitamin-D dose-dependently decreased hydrogen peroxide-induced endothelial cell oxidative stress and apoptosis, which were further inhibited by IGF in the presence of low, but not high vitamin-D concentration.(8)

Aim of the work:

We aimed to study possible effect of vitamin-D status on carotid intimal medial thickness, in relation to insulin like growth factor level.

Subjects and methods:

Study design:

This is an observational study, which was conducted on 100 healthy individuals selected from Ain Shams University from January 2014 to June 2014. They were divided into the following groups: **Group-I:** 50 female and **Group II:** 50 male, aged between 30-40 years ,after informed oral and written consents from all subjects)

Exclusion criteria:

Include patients with type 1 & 2 DM, hypertensive patients, post-menopausal females, obese patients [BMI (body mss index) > 30%], patient on vitamin-D replacement therapy, patients with primary or secondary dyslipidemia and smokers.

All subjects were subjected to the following:

1-Clinical assessment:

Full history taking, body-mass index (BMI) [calculated by the Quetelet formula (weight in kilograms divided by the square of height in meters)], clinical examination (including waist circumference and blood pressure).

2-Laboratory investigation

Biochemical assays included the following laboratory investigations: (routine biochemical assays including lipid profile, Ca,PO4, alkaline phosphatase, creatinine as well as fasting IGF, PTH) using automated standard laboratory techniques, 10 ml of venous blood samples were drawn by venipuncture after 12-14 hours fasting.

Samples were withdrawn on EDTA tubes for PTH assay and plain chemistry tubes for the rest of analytes. Serum and plasma were separated by centrifugation. Lipid profile, calcium, phosphorus. alkaline phosphatase and creatinine were measured by routine automated standard laboratory techniques immediately after centrifugation. For 25 OH vit-D and fasting IGF-1, serum samples were stored at -20° till analysis. PTH was assayed immediately after centrifugation by the two-site chemiluminescent Immulite 2000 assay (Biermann, Bad Nauheim, Germany). Reference interval for PTH is10-65 pg/mL.
IGF-1 was assayed using enzyme linked immunosorbent assay (ELISA), (catalogue number 4140; DRG diagnostics, Springfield, New Jersey, USA). Reference interval for IGF-1 is 70-240 ng/ml.
25 OH vit-D was measured using ELISA, (catalogue number 5396; DRG diagnostics, Springfield, New Jersey, USA). Reference range for 25-OH Vitamin-D is 10-65 ng/ml.

3-Radiological assessment:

Carotid artery intimal-medial thickness (IMT) .

A carotid Doppler test is used to detect degree of narrowing of the arteries in the neck (the carotid arteries) that supply blood to the brain. During this test, these arteries are visualized using high-frequency sound waves (ultrasound) for evidence of plaque (blockages). B- mode Ultrasonography of the left and right common carotid arteries and the internal carotid artery was performed, and IMT was determined, as described by O'Leary et al (9) .Four standardized images were obtained bilaterally for each subject: 1 image of the common carotid artery, 1 cm proximal to the dilation of the carotid bulb at a lateral angle and 3 images of the internal carotid artery at the site of maximal thickness in 3 angles (anterior, posterior, and lateral). The common carotid artery IMT score was calculated as the mean of the left and right measurements of the common carotid artery IMT. The internal carotid artery IMT score was determined as the mean of the 6 internal carotid artery IMT measurements.

The many measures of intima–media thickness were summarized in two variables, one for the common carotid artery and one for the internal carotid artery. The maximal intima–media thickness of the common carotid artery and of the internal carotid artery was defined as the mean of the maximal intima–media thickness of the near and far wall on both the left and right sides.

A composite measure that combined the maximal common-carotid-artery intima–media thickness and maximal internal-carotid-artery intima–media thickness was obtained by averaging these two measurements after standardization ,it is considered increased when more than 1.05cm.

Statistical analysis:

All data were analyzed using software (version 11, SPSS Inc., Chicago, Illinois). Baseline characteristics are presented as mean ± standard deviation for the continuous variables, and as frequency and percentage for the discrete ones. Correlation between variables was examined using the Pearson's correlation coefficient. P value <0.05 was considered statistically significant.

Results

Such study was conducted on 100 healthy individuals from Ain Shams University over 6 months duration being divided equally into 2 groups: female group: group-I and male group: group- II, aged between 30-40 years.

Besides full clinical assessment, all subjects were subjected to the following laboratory investigations: (routine biochemical assays including lipid profile, Ca,PO4, alkaline phosphatase, creatinine as well as fasting IGF, PTH) in addition to B-mode ultrasonography of common carotid arteries and the internal carotid artery bilaterally to assess carotid artery intimal-medial thickness (IMT).

Demographic data of both groups showed that there were almost comparable data values except for notable difference regarding PTH & alk. Phosphatase levels being higher among female group **(78±35.7,133.6 ±52.4. respectively)**, and also higher Ca, vitamin-D & IGF-1 levels among male group **(9.1±0.4, 36.9±10, 192±41.5, respectively)**. ***Table-1***

Table-1:Demographic data and Laboratory results of the studied groups.

	Male group		Female group	
	Mean	**SD**	**Mean**	**SD**
Age/years	35.5	3.2	35.5	3.4
BMI	24.5	2.5	24.3	2.7
Calcium mg/dL	9.1	0.4	8.7	0.4
Po4 mg/dL	3.6	0.5	3.1	0.4
Alk. Phos U/L	122.6	30.6	133.6	52.4
PTH pg/ml	50.0	21.1	78.0	35.7
Vit-D ng/ml	36.9	10.1	26.9	12.6
Cr mg/dL	0.9	0.2	0.7	0.2
Alb g/dL	4.1	0.2	4.1	0.2
IGF-1 ng/ml	192.0	41.5	166.9	46.4
CIMT/cm	0.9	0.1	0.9	0.2
T.chol mg/dL	182.0	18.3	181.3	22.6
TGS mg/dL	102.1	19.6	115.0	20.2
LDL-C mg/dL	119.0	17.7	110.9	20.2
HDL-C mg/dL	43.9	5.1	51.0	5.9

Comparison between those with high CIMT among the 2 groups, (being 5 males & 6 females), regarding other clinical and laboratory parameters showed high statistically significant difference (**P <0.001)** regarding Ca, Po4, vitamin-D level and IGF-1, being higher values **(9.3±0.2, 3.8±0.4, 50.8±5.6 , 225.2±13.6 respectively)** among affected males compared to affected females while higher alkaline phosphatase and PTH values were found among affected females (**191.7±10.6, 119±34.6 respectively)** compared to affected males ***Table-2***.

Table-2: Comparison between high CIMT subjects:

	Affected Sex				T	P Value
	Male (N=5)		Female (N=6)			
	Mean	SD	Mean	SD		
Age/years	37.2	2.8	35.3	3.7	0.934	.375
BMI	23.5	2.0	26.4	2.4	2.093	.066
Calcium mg/dl	9.3	0.2	8.3	0.2	8.038	.000
PO_4 mg/dl	3.8	0.4	2.7	0.2	5.901	.000
Alk. Phos U/L	127.0	17.4	191.7	10.6	7.587	.000
PTH pg/ml	38.6	6.7	119	34.6	5.076	.001
Vit-D ng/ml	50.8	5.6	12	1.8	16.086	.000
Cr mg/dl	0.9	0.2	0.7	0.2	1.488	.171
Alb g/dl	4.2	0.3	4.1	0.2	0.951	.366
IGF1 ng/ml	225.2	13.6	158.8	41.0	3.439	.007
CIMT /cm	1.3	0.0	1.3	0.0	0.634	.542
T.chol mg/dl	187.2	18.8	176.5	17.3	0.983	.351
TGS mg/dl	95.2	21.3	122.0	22.9	1.991	.078
LDL-C mg/dl	118.6	17.5	110.5	19.5	0.717	.491
HDL-C mg/dl	45.0	4.2	47.8	10.0	0.588	.571

Comparison between those with low vitamin-D levels among the 2 groups, (being 6 males & 25 females), regarding other clinical, laboratory & radiological parameters showed statistically significant difference **(P<0.01)** regarding age, PTH and CIMT, being greater age, PTH,CIMT (**36.4 ±3.2, 108 ±23.8, 1±0.2 respectively)** among affected females compared to affected males(**94.3±8.7, 0.9±0.1 respectively).** ***Table -3***

Correlation of CIMT and vitamin-D levels with other clinical and laboratory variables showed statistically significant positive correlation of CIMT among females regarding BMI, PTH ,alk. Phosphatase (r**=0.402,0.440,0.465,P=0.004,0.001,0.001 respectively)** & statistically significant negative correlation regarding Ca,PO4,vitamin D(**r=-0.480,-0.432,-0.606,P=.000,.002,0.000 respectively)** .In males CIMT showed highly statistical significance positive correlation with vitamin D **(r=0.437,P=.001**),and IGF-1**(r=0.306,P=0.031).**

While vitamin D correlation showed positive statistically significant correlation among males regarding Ca and IGF-1**(r=0.566,0.431,P=0.000,0.002)** and negative statistically significant one regarding PTH(**r=-0.0463, P=.001),** As regards females, vitamin-D levels showed positive statistically significant correlation regarding Ca and Po4 **(r=.789,.705,P=0.000 of both respectively),** on the other hand there were negative statistically significant correlation regarding PTH, BMI and alk. Phosphatase **(r=-0.766,-0.444,-0.759,P=.000,.001,.000).** ***Table 4***

Table-3: Comparison between low Vitamin D.

	Affected Sex				T	P Value
	Male (N=6)		Female (N=25)			
	Mean	SD	Mean	SD		
Age/years	33.7	2.4	36.4	3.2	2.281	.046
BMI	27.4	2.7	25.4	2.8	1.605	.148
Calcium mg/dl	8.5	0.1	8.4	0.3	1.336	.203
PO_4 mg/dl	2.7	0.3	2.8	0.2	0.549	.603
Alk. Phos U/L	181.0	9.9	181.2	23.9	0.032	.975
PTH pg/ml	94.3	8.7	108.6	23.8	2.411	.024
Vit-D ng/ml	15.2	1.2	15.2	2.5	0.048	.962
Cr mg/dl	0.9	0.2	0.7	0.2	1.860	.109
Alb g/dl	4.1	0.2	4.1	0.2	0.195	.850
IGF1 ng/ml	182.8	43.8	168.3	46.7	0.719	.492
CIMT/cm	0.9	0.1	1.0	0.2	2.642	.014
T.chol mg/dl	177.2	13.0	188.2	23.6	1.554	.142
TGS mg/dl	105.3	24.5	117.4	20.4	1.121	.300
LDL-C mg/dl	112.7	20.0	118.8	21.7	0.663	.526
HDL-C mg/dl	45.2	6.1	50.0	6.5	1.728	.123

Table-4: Correlation of vitamin-D/CIMT with other parameters.

	CIMT				Vit D			
	Male		Female		Male		Female	
	R	P Value	R	P Value	R	P Value	R	P Value
CIMT/vit. D	**.437****	**.001**	**-.606****	**.000**	**.437****	**.001**	**-.606****	**.000**
IGF1 ng/ml	.306*	.031	-.089	.539	.431**	.002	-.150	.300
Age/years	.076	.600	.099	.492	.190	.187	-.217	.130
BMI	-.014	.923	.402**	.004	-.247	.084	-.444**	.001
Calcium mg/dl	.103	.475	-.480**	.000	.566**	.000	.789**	.000
Po4 mg/dl	.039	.786	-.432**	.002	.284*	.045	.705**	.000
Alk. Phos U/L	.087	.546	.465**	.001	-.313*	.027	-.759**	.000
PTH pg/ml	-.134	.352	.440**	.001	-.463**	.001	-.766**	.000
Cr mg/dl	.159	.270	-.122	.400	-.031	.832	-.012	.936
Alb g/dl	.081	.575	.038	.793	-.013	.929	-.231	.107
T.chol mg/dl	.038	.794	-.009	.949	.087	.548	-.199	.166
TGS mg/dl	.065	.655	.010	.942	.010	.945	-.108	.455
LDL-C mg/dl	-.081	.575	.009	.952	-.004	.977	-.287*	.043
HDL-C mg/dl	-.038	.796	-.176	.222	.058	.688	.088	.542

About 31% of studied population had low vitamin-D level as well as 11% of them had high CIMT ***Table-5***. There was statistically high significant negative correlation between vitamin-D and CIMT among female group and statistically significant positive correlation between both variables among male group ***fig-1 & 2***.

Table-5: Frequency of vit-D level, and CIMT aberrations.

VitD		
	Frequency	**Percent**
Deficient	31	31.0
Normal	69	69.0
Total	100	100.0

CIMT		
	Frequency	**Percent**
Normal CIMT	89	89.0
High CIMT	11	11.0
Total	100	100.0

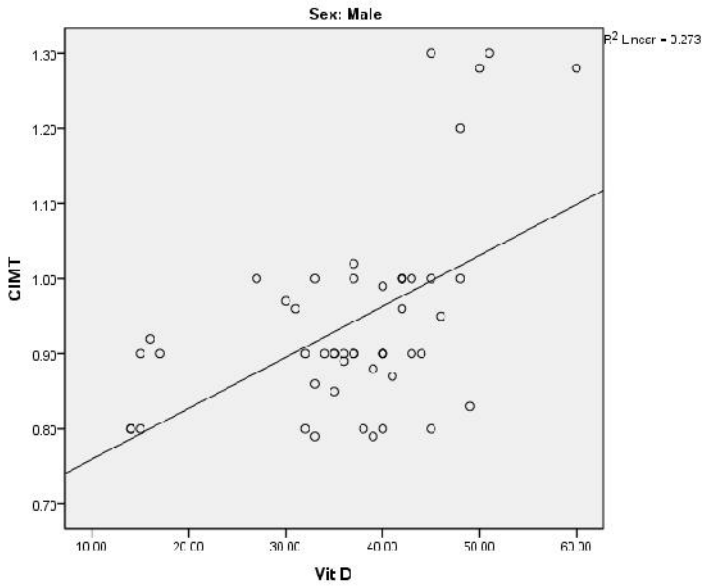

Fig-1: Correlation between CIMT and Vitamin D level among males

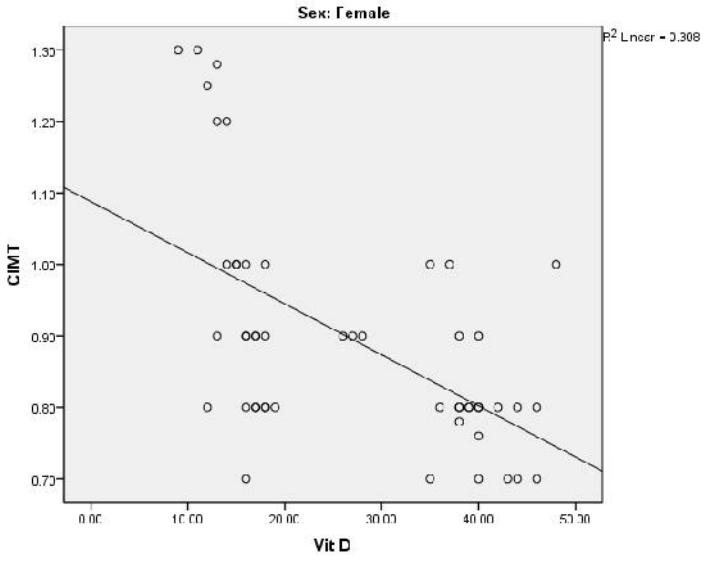

Fig-2: Correlation between CIMT and Vitamin D level among females.

Discussion

Vitamin D deficiency has been linked to an increased risk of hypertension, diabetes, congestive heart failure, peripheral arterial disease, myocardial infarction, stroke, and related mortality, even after adjustment for traditional cardiovascular risk factors. Accumulating evidence from experimental, clinical, and epidemiological studies suggests that vitamin D may also be associated with several incidences of vascular dysfunction, including the development and progression of atherosclerotic cardiovascular disease. (10).

However, not only vitamin D but also PTH has been inconsistently found to be associated with early signs of atherosclerosis, such as increased carotid intima-media wall thickness (IMT) determined with B-mode ultrasound (12,11).

As, The increased carotid intima-media thickness is an established feature of the age related vascular phenotype and is interrelated with atherogenesis (13),this study aimed to investigate possible effect of vitamin-D on carotid intimal thickness since it has been implicated in the vascular protection, in relation to insulin like growth factor level.

This study showed a significant higher levels of Ca, vitamin D and IGF-1 levels among male group collectively while showing higher levels of PTH among female group collectively. And also, we found a significant positive correlation between both PTH and CIMT in the female group (r=0.0440,P=.001), and a non significant correlation between both parameters in the male group(r=-0.0134,P=.352) and this was consistent with Choi et al. (14) who observed a direct association between PTH and carotid IMT independent of established cardiovascular risk factors among postmenopausal women. So that , a role for PTH in the development of cardiovascular disease has been suggested by several studies (15,16).

Mirella et al 2005, found that Low IGF-1 concentration has been associated with many cardiovascular risk factors including age, adiposity, high TG, high fasting insulin and C-reactive protein. (17).And this was in agreement with Shai et al ,2011 who found , an inverse association between IGF-1 concentrations and carotid intima-media thickness in humans (18,19). However, other authors reported a positive correlation between circulating IGF-1levels and CIMT (20).

The findings of our study reported that in males there was positive significant correlation between CIMT and IGF-1 but non significant negative correlation in the females (r=.0306,-0.089,P=.031,0.539 respectively). Close to our results, Pietra et al (21) found a significant positive correlation between CIMT and IGF-1 which was found in the male group of our study.

On contrast to this finding , Mirella and his colleagues stated the IGF-1 concentrations were positively correlated with the mean CIMT in women (r=0.127,P=0.009) whereas the association in men was weaker and negative (r=-0.088,P=0.034) .(17)

Pre-clinical studies have shown that vitamin D can affect the synthesis and activity of IGF-1 at the tissue level, as well as the amount of IGF-1 in the circulation (7). Our study also showed that vitamin D is significantly correlated in a linear positive correlation with IGF-1 in the male group(r=.431,P=.002) while there was negative non significant correlated in the female group(r=-0.150,P=.300). Also, Wu-Wong and his colleagues found that vitamin D up-regulates IGF-1 mRNA in human coronary artery smooth muscle cells (22).

Regarding relation of vitamin D and CIMT ,our study showed a statistically significant positive correlation between CIMT and 25(OH) vit D in the studied male group(r=0.437, P=.001)but found a significant inverse correlation between the same two parameters in the studied female group(r=-0.606,P=.000).This previous finding regarding the female group was close to those of Jared et al (23) who found a significant inverse stepwise association between serum 25(OH)D levels and carotid IMT independent of age, sex. Differences in the selection criteria and demography of the study populations may be responsible for the contradictory findings with our male group.

Also, Targher et al. (11) reported an inverse association between 25(OH)D and IMT among 390 diabetic patients, but no association was observed in another study of 109 postmenopausal women (14).

Dobnig et al.(24) recently showed that low levels of 25(OH)D was associated with higher rates of all-cause mortality, even after adjustment for the other vitamin D metabolite and PTH. Furthermore, the mortality risk was greatest among those in the bottom quartile of 25(OH)D and $1,25(OH)_2D$, suggesting a possible synergistic effect for those deficient in both vitamin D metabolites.

On the other side, Ameri et al,2014 stated that circulating IGF-1 has a vasoprotective effect only when vitamin D levels are low as they hypothesized that hydrogen peroxide –induced endothelial cell oxidative stress and apoptosis are inhibited by IGF-1 in the presence of low but not high vitamin D concentration(8).

Experimental evidence indicates that IGF-1 inhibits endothelial dysfunction and vascular inflammation that occur with aging and drive atherosclerosis development and progression (25).

So, Vitamin D, has been reported to be beneficial for the vasculature by of a number of mechanisms, such as preservation of endothelial function and integrity, and inhibition of arterial inflammation and immune activation (24). Vascular cells express the vitamin D receptor and the 1a-hydroxylase enzyme that converts 25(OH)D into the hormonally active metabolite 1,25(OH)2D (26,27).

So, Endothelial dysfunction could be impaired in otherwise healthy vitamin D deficient subjects as represented by increased carotid intimal thickness and this finding is evident in females in comparison to male in our study but the exact mechanism is not well established yet.

Conclusion

The exact mechanism by which vitamin D may influence the atherosclerotic disease process has not yet been completely elucidated. But vitamin D seemed to play important role in the pathophysiology of atherosclerosis , but it is still unclear at what stage(s) in the atherosclerotic disease process vitamin D may exert its effects.

Recommendation:

Large-scale, well-conducted, placebo controlled clinical trials testing the relation of vitamin D to atherosclerosis and the efficacy of vitamin D supplementation in delaying, slowing, or reverting the atherosclerotic disease process should be studied.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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