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Assessment of Klotho Protein and Fibroblast Growth Factor 23 On the Progression of Atherosclerosis In Vitamin D Deficient Egyptian Patients.

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ABSTRACT

Vitamin D deficiency is associated with several diseases. We aimed to investigate the relationship between plasma vitamin D level, interleukin 17 (IL-17), rennin, Klotho protein , Fibroblast growth factor 23 (FGF-23) and serum nitric oxide (NO) levels in atherosclerotic patients. The study included 40 atherosclerotic patients and 15 controls. The patient group was divided into two subgroups according to plasma vitamin D level: atherosclerotic with vitamin D deficiency (VDD) and atherosclerotic with vitamin D insufficiency (VDI) group. The study included 40 male patients with age above 40 years with atherosclerosis. Clinical (coronary angiography) as well as biochemical investigations including the determination of plasma vitamin D, IL-17, FGF-23, rennin, klotho, serum NO, some lipid parameters, serum calcium and phosphorus levels were determined. All the previous parameters were measured according to the appropriate methods. Significant decrease of plasma vitamin D level in the patient groups VDD (7.20 ±1.02) and VDI (25.35± 0.89) and plasma klotho protein in VVD(290.7 ±17.12) and VDI (342 ± 14.06). Opposite trend was found with plasma FGF-23 in VVD group (134.2± 4.82) and in VDI group (64.4± 2.66) that increased significantly. plasma vitamin D, Klothoprotein and FGF-23 may be used as predictive parameters for atherosclerosis.

Keywords: Atherosclerosis, Vitamin D, Klotho, FGF-23

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INTRODUCTION

Cardiovascular diseases (CVDs) are the first cause of death globally. Of these deaths, an estimated 7.4 million were due to coronary heart disease (CHD) and 6.7 million were due to stroke.[1]

Atherosclerosis is recognized as an inflammatory disorder of medium and large arteries it is a progressive disease characterized by the accumulation of lipids and fibrous elements in the arteries [2]. Atherosclerosis is a chronic proinflammatory disease that affects arteries and leads to coronary artery disease(CAD) and late complications including stroke and acute coronarysyndrome (ACS). [3]

The disease is initiated by the activation of the endothelium/endothelial cell (EC) dysfunction by accumulation of low density lipoprotein (LDL), which issubsequently modified (e.g. oxidized), together with other atherogenic factors.[4]

Risk factors such as high fat diet, smoking, high blood pressure, diabetes and obesity have shown to increase the chance for developing atherosclerosis.[5-6]

The routine methods for the detection of atherosclerosis mainly include the determination of some lipid parameters such as total cholesterol (TC), triacylglycerol (TAG), high density lipoprotein cholesterol (HDL-C), andlow density lipoprotein cholesterol (LDL-C) concentrations[7], also the coronary angiography was performed using standard techniques.[8]

Vitamin D deficiency is emerging as a risk factor for CVD development,new evidences suggest that low levels of vitamin D may increase cardiovascular risk.[9]

The interaction of the active form of vitamin D (1.25-dihydroxyvitamin D_3) with the vitamin D receptor (VDR) has a wide variety potential cardiovascular benefits including reducing production of rennin, causing relaxation of vascular smooth muscle cells and decreasing the generation of atherosclerotic forming foam cells.[10]

The fibroblast growth factor 23 (FGF-23) is a hormone that is secreted primarily by osteocytes and to a lesser extent by osteoblasts . It is involved in the regulation of phosphorus homeostasis, vitamin D metabolism, and bone mineralization. specifically, it induces urinary phosphorous excretion, inhibits activation of calcitriol [$1,25(OH)_2D$], and suppresses parathyroid hormone (PTH) synthesis.[11]FGF-23 may influence cardiovascular risk through the chronic kidney disease(CKD) or vitamin D pathways.[12]

Klotho was identified as an aging-suppressor protein while disruption of the Klotho gene results in accelerated aging and shortened lifespan.[13]Also, klotho was suggested that it may suppress inflammation to protect the vascular wall integrity.[14]Therefore, Klotho has been suggested as a master regulator of CVD, with a potential role in the pathogenesis of atherosclerosis.[15]

Rennin also plays an important role in the advancement of atherosclerosis by influencing on physiology of the endothelium, inflammatory reactions, thrombosis, and oxidant injury.[16]

Endothelium-derived relaxing factor (EDRF) or NO plays an important role in preserving the endothelial vasodilatation and inhibiting the vasoconstriction triggered by angiotensin II and endothelin.[17]

For early detection of atherosclerosis, systematic screening and appropriate management are needed to evaluate the progression of atherosclerosis in patients. In accordance, this study was constructed to evaluate the clinical usefulness of klotho protein, FGF-23 and vitamin D as prognostic biomarkers in the progression of atherosclerosis in atherosclerotic Egyptian patients. As well as correlating them with some inflammatory markers.

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METHODS

Subjects

55 male subjects were enrolled in this study; 15 normal control subjects and 40 patients with atherosclerosis from El-Demerdash hospital were included in this study. Written informed consent was obtained from all patients after full explanation of the procedure used. The diagnosis of atherosclerotic patients was performed according to the world health organization (WHO) criteria.

The atherosclerotic patients were subdivided into two groups: atherosclerotic patients with deficiency in plasma vitamin D level (10-20 ng/ml) (20 patients) and atherosclerotic patients with insufficient plasma vitamin D level (21-29 ng/ml) (20 patients). The patients were treated with different drugs as follow: Antihypertensives (Lasix, Aldactone, Concor, Ezapril, Effox, Capoten, Altiazem, Vastrel, Tenormin, Tritace), hypoglycemic agents (Diamicron, Amaryl), anticoagulants (Aspocid) and drugs for heart diseases (Metacardia, Nitromack, Plavix, Myogrel, Plavictonal, Dinitra, Clopex). None of the control subjects were taking any medications.

Exclusion criteria of the subjects were as follows: open heart operations, pregnancy, malignancy and history of drug abuse. Before starting, informed consent was obtained from all participants. Approval was taken from Ain Shams University.

Methods

Standardized questionnaire was used for history of diseases, medication and age on each subject. blood samples (approximately 5 ml) were collected on EDTA labeled tubes ,centrifuged at 2000- 3000 rpm for 10 minutes, plasma collected and aliquoted for the measurements of the levels of plasma vitamin D, IL-17, FGF-23, klotho protein and Rennin.

Another part of blood was collected and left to clot, then centrifuged at 2000 rpm for 10 minutes, sera were separated, aliquoted for the measurements of the levels of serum TC, TAG, HDL-C, LDL-C, calcium (Ca), phosphorus and NO.

Biochemical investigations

Commercial kits were purchased fromDRG Company (USA), Glory Science Company (USA), Spectrum Diagnostic Company (Egypt) and Bio-diagnostic Company (Egypt). plasma vitamin D concentration was determined using a commercially available enzyme linked immunosorbent assay (ELISA) kit (DRG Company, USA) based on the method described by Pilz et al., (2011).[18]

plasma IL-17 and FGF-23 concentrationswere determined using a commercially available ELISA kits (Glory Science Company, USA) based on the method described by Yu et al., (2011) andSmith et al.,(2013) respectively .[19-20]

Plasma Rennin concentration was determined by the method of Carey & Padia.,(2008) using acommercial ELISA kit (DRG Company, USA).[21]

plasma klotho protein concentration was determined using a commercially available ELISA kit (Glory Science Company, USA) based on the method described by Yamazaki et al., (2010).[22]

Lipid parameters including TC, TAG, HDL-C and LDL-C were detected by biochemical auto- analyzer. Serum Ca, phosphorus and NO were determined according to Hasegawa et al., (1990), young,(1991) and Montgomery & Dymock, (1961) respectively.[23-24-25]

statistical analysis

statistical analysis was performed using the Statistical Package for the Social Science (SPSS). Data were presented as mean ± S.E. the data were analyzed by one way analysis of variance (ANOVA). A P value

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<0.05 was considered statistically significant. Pearson's correlation coefficient analysis was used to determine the correlation between the different parameters.

RESULTS

The results of the current study were summarized in table(1). It showed that plasma vitamin D, klotho protein and serum NO levelswere highlysignificant decreased(p<0.001) in VDD and VDI patient groups in comparison to control group. On the other hand, the results of the current study showed that plasma FGF-23, IL-17 and rennin concentrationswere highly significant increased(p<0.001) in the VDD and VDI patient groups in comparison to control group. Data in table (1) also showed that, the both studied groups have significant increase in the levels of serum TC, TAG , LDL-C and calcium. while, showed significant decrease in serum HDL-C and phosphoruslevels.

Table (1): Biochemical parameters for subjects included in the study

parameters	Groups		
	Control	Atherosclerotic, Vitamin D deficiency (VDD)	Atherosclerotic, Vitamin D insufficiency (VDI)
Number of subject	15	20	20
Age(year)	57.9± 2.1	56.6± 2.1	56.85± 1.25
SBP(mmHg)	122.93±2.2	146.2±2.1	143.8±1.0
DBP(mmHg)	76.74 ±2.53	92.2± 0.99	91.65± 0.41
Vitamin D(ng/ml)	47.53± 2.78	7.20±1.02	25.35±0.89
IL-17(ng/L)	7.13±1.19	49.55± 2.49	37.05±2.10
FGF-23(ng/L)	22.67±0.87	134.2±4.82	64.4±2.66
Klotho(U/L)	867.73±27.27	290.7±14.12	342.0±14.06
Rennin(pg/ml)	12.6±0.71	38.35±1.45	27.55±1.022
TC(mg/dl)	145.60±6.34	264.70±7.81	238.45±8.54
TAG(mg/dl)	124.87±4.61	242.51±9.033	229.0 ±10.0
HDL-C(mg/dl)	40.13±2.68	32.20±1.69	34.45±1.80
LDL-C(mg/dl)	69.467±4.05	145.27 ±6.15	142.55±5.18
VLDL-C(mg/dl)	24.97 ± 0.92	39.9±2.54	41.58 ±2.24
Ca(mg%)	7.99 ±0.21	11.21 ±0.54	10.75 ±0.66
Phoshporus(mg%)	3.57 ±0.14	1.27 ±0.07	2.04±0.10
NO(µmol/L)	66.53 ±1.92	30.9±1.12	42.0 ±2.39

Data are expressed as mean ±SE.

IL-17, Interleukin-17; FGF-23, Fibroblast growth factor-23; TC, total cholesterol; TAG, triacylglycerol; HDL-C, high density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol; VLDL-C, Very low density lipoprotein cholesterol; Ca, calcium; NO, nitric oxide:
* Significant from control (P<0.05), ** High significant from control (P<0.001).

Correlation between parameters

Table (2) showed the pearson's correlation coefficients (r) between vitamin D level and some biochemical parameters in the patient groups. There is a significant positive correlation between vitamin D and both klotho protein (r 0.949, r 0.923) and NO (r 0.91and r 0.98) in patient groups VDD and VDI respectively. There is a significant negative correlation between vitamin D and IL-17 (r -0.96, r -0.94), FGF-23 (r -0.95, r -0.91) and rennin (r -0.91, r -0.97)in patient groups VDD and VDI respectively.

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Table (2): pearson's correlation coefficient (r) between vitamin D and some of the biochemical parameters in the patient groups

parameters	Groups			
	Atherosclerotic, Vitamin D deficiency(VDD)		Atherosclerotic, Vitamin D insufficiency(VDI)	
	r	P value	r	P value
IL-17 (ng/L)	-0.955	<0.001	-0.935	<0.001
FGF-23 (ng/L)	-0.947	<0.001	-0.909	<0.001
Klotho (U/L)	0.949	<0.001	0.923	<0.001
Rennin (pg/ml)	-0.905	<0.001	-0.969	<0.001
NO (µmol/L)	0.91	<0.001	0.983	<0.001

Pearson's correlation (r), p<0.001: highly significant

Fig (1) illustrates the percent change of IL-17, FGF-23 and rennin in the patient groups. IL-17, FGF-23 and rennin showed marked increase in patient groups (594.66, 492.05 and 204.37 respectively) in the VDD group and (419.42, 184.11 and 118.65 respectively) in the VDI group.

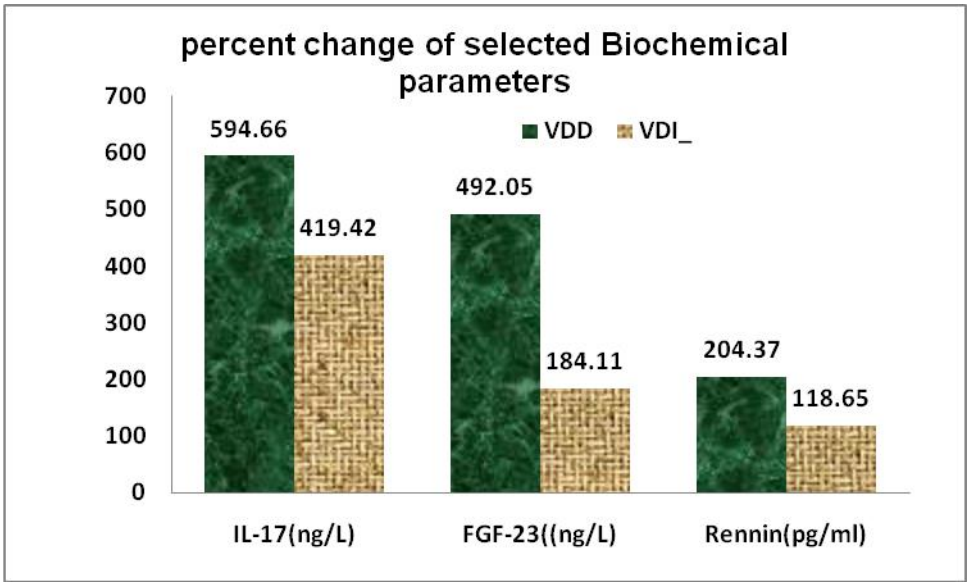

Figure (3): percent change of IL-17, FGF-23 and Rennin in VDD and VDI patient groups.

DISCUSSION

Atherosclerosis is a multi-factorial disease, and is the basis for stroke, CHDand myocardial infarction, which remain the leading cause of mortality. [26] The onset and development of CVDs are characterized by atherosclerosis and atherothrombosis, of which the most important physiopathological changeis the presence of endothelial dysfunction . [27]

Early identification of atherosclerosis is of critical importance to improve patient outcomes. The current study investigated the predictive value of plasma vitamin D, FGF-23 and klotho protein, where plasma vitamin D, FGF-23 and klotho protein were evaluated as important biochemical markers associated with atherosclerosis.

Results of the present study reveals that plasma vitamin D level dramatically decreased in both studied groups.

Since vitamin D modulates the expression of sirtuins, i.e. regulatory proteins involved in oxidative stress and atherogenesis.[28] Also vitamin D may suppress the pathological immune process of atherosclerosis by inducing proliferation and/or differentiation of regulatory T-cells. [29]

It was found that decreased levels of vitamin D alters a range of pathways in ECs, extracellular matrices, and vascular smooth muscle cells (VSMCs), which are implicated in atherosclerosis pathogenesis, also hypovitaminosis D promotes atheroma formation so vitamin D deficiency highlighted as a promoter of atherosclerosis. [10]

Results of the current study were consistent with Eva et al[29]who reported thatvitamin D exerts a variety of favourable effects on endothelialdysfunction, VSMC proliferation and migration, and calcification,as well as on the inflammatory/immune process ofatherosclerosis; moreover, it exerts beneficial effects againstsystemic conditions that promote atherosclerosis such as insulin resistance, dyslipidemia, rennin-angiotensin-aldosterone system (RAAS),and consequent hypertension, therefore suggesting a potentialtherapeutic role.

The current study shown that plasma klotho protein was highly significant decreased in the patient groups.

Klotho protein is involved in the regulation of NO production,integrity and permeability of endothelium. Lower expression of klotho protein in humans reduces NO production and increases the level of endothelin-1 (ET-1) and inflammatory medium. [30]

The previous studies verified that klotho enhances blood IL-10 level suggesting that klotho may suppress inflammation process and protect the vascular wall integrity .[14]

Results of this study were in agreement with Abd Allah et al[31]who explained that Klotho protein exertsstrong cardio protective effects. it protects against vascular calcifications higherKlotho protein levels have been related to a lower incidence ofmortality and CVD Interestingly, inhibition of Klotho protien expressionin aortic VSMCs resulted in acceleratedcalcification of these cells.

On the other hand results of the present study reveals that plasma FGF-23 levels was highly significant increased in both patient groups.

The pro-inflammatory stimuli enhance FGF-23 secretion from bone.[32] FGF-23 increases the reabsorption of calcium and sodium, which may indirectly contribute to vascular calcification. FGF-23 is mediated indirectly through renal calcium retention and suppression of vitamin D hormone production, which may in turn promote endothelial dysfunction [31].

The results of current study were consistent with Erben [33] who reported that FGF-23 is far more than only aphosphaturic bone-derived hormone. Rather, FGF23 has emergedas a pleiotropic endocrine and auto-/paracrine factor not onlyinvolved in phosphate homeostasis, but also in calcium and sodiummetabolism, in bone mineralization as well as in the development of cardiac hypertrophy and cardiovascular diseases.

In the present study it was found that plasma IL-17 concentrations were highly significantly increased in the patient groups.

Since IL-17 producing T cells was detected within atherosclerotic coronary plaques, that suggesting a pro inflammatory effect of IL-17 on VSMCs. [34]Also, it was showed that oxidized LDL promoted IL-6 production, which then induced Th17 cell differentiation. [35]Also, reactive oxygen species (ROS) associated with atherosclerosis was shown to induce cyclic adenosine monophosphate (cAMP) response element–bindingprotein–dependent IL-17 suggesting a contribution of IL-17 pathway to vascular inflammation . [36]

Results of the present study reveals that plasma rennin levels was highly significant increased in both patient groups.

Rennin–angiotensin system (RAS) or the rennin–angiotensin–aldosterone system (RAAS) is a hormone system that is involved in the regulation of the plasma sodium concentration and arterial blood pressure. [37]

Since the RAAS is the major regulator of blood pressure. It is directlyinvolved in the pathogenesis of atherosclerosis.

As vitamin D is apotent endocrine suppressor of the RAAS,it suppresses both rennin gene expression via a vitaminDresponsive element in the promoter of the rennin and prevent the development of hypertension.[38]

Results of the current study were consistent with Carvalho & Sposito [38] and Gunta et al [39]Who reported that RAAS is a key player in atherosclerosis development.

Results of this study showed that serum NO concentration was highly significant decreased in both studied groups.

Since NO is a potent vasodilator and anti-atherogenic agent, that is produced in the endothelium from the amino acid L-arginine via the action of endothelial nitric oxide synthase (e-NOS).[40] It plays a protective role by suppressing abnormal proliferation of VSMCs following various pathological situations including atherosclerosis.[41]Vitamin D may suppress the pathological immune process of atherosclerosis by enhancing the production of NO.[42]

CONCLUSION

In conclusion, due to the high incidence of atherosclerosis in Egyptian population, there was a great need to search for a more accurate markers for screening the progression of atherosclerosis. Our data supported the use of plasma vitamin D, FGF-23, IL-17, klotho, rennin and serum NO levels as important markers for atherosclerosis detection. Plasma FGF-23 and rennin were the most sensitive markers in VDD patient group, while plasma IL-17 was the most sensitive marker in the VDI patient group.

Conflict of interest

Authors have no conflict of interest.

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