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Modulation of endogenous angiotensin II, systolic, and diastolic blood pressure in hypovitaminosis D patients with cardiovascular risk after oral vitamin D3 supplementation

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ABSTRACT

Considerable evidence suggests that a large percent of the population have low vitamin D levels, which may affect the cardiovascular system (CVS) adversely. The possible mechanism includes modulating adaptive immunity and vascular inflammation, maturation, and differentiation of cardiomyocyte modulating response in the vascular endothelial. This study is designed prospectively to evaluate the potential effectiveness of oral vitamin D3 as a therapeutic strategy in the primary prevention for cardiovascular risk in subjects with high cardiovascular risk score and hypovitaminosis D. Forty individuals at high risk for cardiovascular diseases and vitamin D deficiency (< 20 ng/ml) were selected. The candidate was assigned to receive vitamin D3 (100000 IU) orally every 2 weeks for 8 weeks. The measurement includes Plasma 25-hydroxyvitamin D level, serum Angiotensin II, serum creatinine, serum GPT, estimation of lipid profile, in addition to the obesity status through measuring. The WHO/ISH risk prediction charts were used to assess the risk score for cardiovascular diseases. The result showed a highly significant decrease (P<0.01) in the serum Ag II in the intervention group on oral vitamin D3 supplement, a highly significant increase (P<0.01) in serum endogenous vitamin D status, and a highly significant decrease (P<0.01) in both systolic (SBP) and diastolic blood pressure (DBP). No significant changes in FBG, lipid profile, and atherogenic index in both study groups. The effect of vitamin D3 supplementation on both angiotensin II level and endogenous vitamin D levels was significant regardless of age group, gender, BMI, and smoking status.

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of disability and premature death in the world. It kills about 17.7 million people every year, which equals 31% of all global deaths (Organization, 2003). In people with the established CVDs, risk factor modification can reduce clinical events and premature death in addition to those who are at high CVRdue to one or more risk factors (Organization, 2007a). Risk assessments are used to determine the possibility of patient developing heart diseases, such as stroke or heart attack in the future (Goff *et al.,* 2014). Several predicting factors can be used to evaluate the patient's risk of developing heart disease in the next ten years (Yamout *et al.,* 2014).

Evidence-based guidance can reduce the incidence of first and recurrent clinical cardiovascular disease, accordingly, cardiovascular risk can include two categories; People with risk factors which

have not yet developed clinically manifest cardiovascular disease (primary prevention), And people with established CHD, CVD or peripheral vascular disease (secondary prevention) (Vandvik *et al.,* 2012).

Several risk assessment methods and calculations are available (Ulusoy, 2013), and the methods reliable one to assess Eastern Mediterranean population for risk assessment calculations is the one provided in the WHO/ISH pocket guidelines for assessment and management of cardiovascular risk, which depend on age, sex, blood pressure, smoking status, total blood cholesterol and presence or absence of diabetes mellitus for 14 WHO epidemiological sub-regions risk prediction charts (Organization, 2007b).

The presence of risk is not essential for treatment indication for individuals belonged to the high-risk category, all of them need intensive lifestyle interventions and appropriate medications (Organization, 2003).

The CVD risk may be higher than indicated by the charts if previously on antihypertensive therapy, a first-degree relative of premature CHD or stroke, premature menopause, obesity exist. In addition to raised levels of C-reactive protein, fibrinogen, homocysteine, apolipoprotein B, fasting glycaemia, or impaired glucose tolerance microalbuminuria (increases the 5-year risk of diabetics by about 5%). Besides the raised pulse rate, socioeconomic deprivation (Organization, 2005).

Endogenous vitamin D exert beneficial effects on vascular smooth muscle cells (Bouillon *et al.,* 2008), the endothelium (Tarcin *et al.,* 2009), and cardiomyocytes (Zittermann *et al.,* 2008), and has an inverse relationship with plasma renin activity, regardless of baseline renin levels or salt intake (Vaidya and Williams, 2012). The effect of dietary salt on blood pressure is worsened in the presence of vitamin D deficiency since it is correlated with calcitriol synthesis, moreover, vitamin D increase renal plasma flow (RPF), hence decreases mean atrial pressure (Vaidya *et al.,* 2011). Vitamin D (3) therapy in obese hypertensives modified RPF, MAP, and tissue sensitivity to AngII similar to converting enzyme inhibition. Whether chronic vitamin D (3) therapy abrogates the development of diseases associated with excess RAS activity warrants further investigation (Vaidya *et al.,* 2012). Renin-angiotensin-aldosterone activation increase vascular tone and arterial stiffness (Quyyumi and Patel, 2010). The vascular function worsens with a low level of vitamin D, and it improved after six months of vitamin D therapy (Patel *et al.,* 2011). Vitamin D also impacts endothelial cell function, regulating endothelial cell-dependent vasodilation (Sugden *et al.,* 2008).

In a meta-analysis of three cohorts, lower 25 (OH)D was associated with an 80% greater risk of incident hypertension (Harinarayan, 2014).In the NHANES III study, the average systolic blood pressure was nearly 3 mm Hg lower in subjects with the highest quartile of 25 (OH)D compared to subjects in the lowest quartile (Scragg *et al.,* 2007).

Measures of arterial stiffness were inversely correlated with vitamin D status in the Baltimore Longitudinal Study of Aging, in a British multiethnic study, as well as in most studies on patients with diabetes, rheumatological conditions, peripheral arterial disease, and renal insufficiency (Pirro *et al.,* 2012). Thus, current evidence indicates that vitamin D deficiency may promote vascular dysfunction and sustained RAS activation, while sufficient vitamin D levels may afford proximal inhibition (Motiwala and Wang, 2012). However, only a few studies examined the effects of vitamin D therapy on vascular function, and so far results were contradictory (Gepner *et al.,* 2012).

This study is designed to evaluate the potential effect of vitamin D3 supplementation in hypovitaminosis D subjects with cardiovascular risk to be potentially assigned as a primary preventive therapy from cardiovascular diseases.

Patients and Methods

Study design

This study is an interventional prospective randomized controlled, open-label study to evaluate the patients benefit from vitamin D3 supplementation to be assigned as a primary preventive therapy for cardiovascular risk in hypertensive patients with low endogenous vitamin D level. This study was conducted during the period from September 2016 to May 2017.

Patients

Total of 60 subjects were selected during their visit to privet clinic of interventional cardiologist. Both male and female gender was included. Forty candidate patient complete intervention course and 20 were excluded from the study (unable to contact or refuse to follow up)

The ethical agreement of general health directorate in the Governorate was approved, and the candidate patient written consent was taken after a full explanation of the aim of the study and ensures the reliability of the collected information. A direct interview was made with each patient to full fill a checklist asking about the patient's age, gender, weight status, and medical history.

Table 1: Cardiovascular risk assessment of study groups

Study groups	Number of patients	CV risk score	Level of risk
G1	Total (20)		
	12	< 10 %	Low risk
	5	10% - 20%	Moderate-risk
	3	20% - 30%	High risk
G2	Total (20)		
	12	< 10 %	Low risk
	4	10% - 20%	Moderate risk
	4	20% - 30%	High risk

Subjects were enrolled if they had body mass index 30 kg/m^2 or greater, 25 (OH)D less than 25 ng/ml, age range 40-70 years, normal renal function (serum creatinine <1.5 mg/dl). Regarding age, patients were divided into two subgroups; less than 60 years of age, and those who aged 60 years and above. Weight status according to the body mass index (BMI) was divided in to obese (BMI ≥ 30) and nonobese (BMI **<**30). All candidates were assessed to have vitamin D deficiency (**<** 20 ng/ml). The risk of cardiovascular disease was assessed according to WHO risk score (Organization, 2007b)

Subjects were ineligible for enrolment if they had any history of mild to moderate hypertension (defined as an untreated blood pressure of 140/90 to 159/99 mm Hg or the use of any antihypertensive drugs for a diagnosis of hypertension), coronary artery disease, heart failure, cerebrovascular disease, liver disease, history of parathyroid or granulomatous or primary bone disorders, hyper- or hypocalcemia, or any recreational drugs, use of oral or transdermal contraceptives, use of antipsychotic or benzodiazepine or bisphosphonate drugs, or were pregnant or breastfeeding.

The participants were allocated into two main groups:

Group 1 (G1): Include 20 subjects with cardiovascular risk and presented with vitamin D deficiency assigned to receive vitamin D3 (100000 IU) orally.

Group 2 (G2): Include 20 subjects with cardiovascular risk and presented with vitamin D deficiency who do not receive any treatment (control group).

The participants of drug group given D3 oral ampule 100,000 IU every two weeks for 8 weeks (4 ampules totally). Patients advised taking D3 ampule after dilution with 100 ml milk. Control group were checked and investigated in the same way and all participants were asked to come again for checking after 8 weeks when the same information was taking and same examination and investigation were done.

Assessment of cardiovascular risk

Following the recommendations for prevention of cardiovascular disease in people with cardiovascular risk factors (according to individual total risk)[3], participants of both study groups are categorized according to the risk score after applying the risk assessment methods and calculations of Eastern Mediterranean population of WHO guideline (Organization, 2007b) (Table 1).

Methods

The patient assessment was done by monitoring the subjective and objective parameters after 8 weeks of treatment. A blood sample was taken to estimate some biomarkers at baseline and after 8 weeks of vitamin D supplementation. The systolic (SBP) and diastolic blood pressure (DBP) was measured using an automatic blood pressure monitor (Rossmax Company). The weight status was checked and the body mass index was calculated. Angiotensin II (Ag II) was estimated using a competitive immunoluminometric assay by using autoanalyzer instruments Maglumi AII· (reference values of Ag II is 25 -125 pg/ml) (Skurk *et al.,* 2001). Vitamin D was estimated using a competitive protein binding assay (2017), and the reference value is 30-60 ng/ml (Holick *et al.,* 2011). The Serum Glutamate Pyruvate Transaminase (GPT), SCr, and lipid profile were estimated using autoanalyzer (Mindray instrument). Glucose also was measured by diagnostic kit (SPINREACT company) according to a method of Borham and Trindoer (1972) (Barham and Trinder, 1972, McDonald *et al.,* 1965). Atherogenic index of plasma (AIP), calculated as log (TG/ HDL-C), with TG and HDL-C expressed in molar concentrations (Harinarayan, 2014). Body Mass Index is a simple calculation using a person's height and weight. The formula is BMI = kg/m^2 where kg is a person's weight in kilograms and m^2 is their height in metres squared.

Statistical analysis

Collected data was introduced into Microsoft Excel 2016 and loaded intoIMB-SPSS V 23 software for statistical analysis. Descriptive statistics were presented using frequency, distribution, tables and

graphs. Analysis of covariance (ANCOVA) test was used to test the significance of the difference between related studied variables at end line time of the study after adjustment of the baseline reading of the same variables.

SHAPIRO- Wilk test was used to test the normality of distribution of studied variables before application of ANCOVA test. A *P*-value less than 0.05 was conceders as discrimination point of significance of results.

RESULTS

Patients demographic and disease characteristics

The results of patient demographic and disease characteristics of 40 patients included 20 females (50%) and 20 males (50%) showed with no significant difference between the study groups of both genders (*P*>0.05). The age range for all patients was between 40-70 year with the mean age of the study groups were (55.35 ± 8.56) years for group 1 (treatment group) patients and (52.3 ± 9.17) years for group 2 (control group) patients. No statistically significant difference found between study groups with respect to age (*P*>0.05).

The mean body mass index (BMI) for group 1and group 2 patients were (30.88 ± 4.7) and (28.83 ± 3.47) kg/m^2, respectively. No statistically significant difference was found between the study groups with respect to the BMI (*P*>0.05). Smoking status in group1represnts 10 (50%) patients were smokers and non –smokers equally. MeanwhileSmoking status in group 2 showed13 (65%)smoker patients and 7 (35%) non-smokers patient.No significant difference found between study groups with respect to smoking status (*P*>0.05).

Moreover, no significant difference was found between both group 1 and group 2 patients in respect to the liver enzyme SGOT (19.05±5.155vs 20.9±4.887)IU/dl, and SCr (0.775±0.055vs 0.750±0.060) (*P*>0.05) respectively, (Table 2).

Effect of vitamin D3 supplement on biochemical markers and blood pressure reading

After adjustment of the baseline means for treatment group and control according to the covariance analysis, Table 3 shows the overall effect of vitamin D3 oral supplement on biochemical markers and blood pressure reading of hypovitaminosis patients with Cardiovascular Risk.

Results showed that vitamin D3 supplements produce a highly significant decrease (p <0.01) in serum Ag II levels in treatment group 1 compared with control group2 where the level reduced after 8 weeks of treatment.

Vitamin D3 supplements produce a highly significant change (p <0.01) in the endogenous serum vit. Dlevelsin group 1compared to group 2 where the level increase after 8 weeks of treatment.

Referred to the results presented in Table 3, there were no significant changes (*p*>0.05) in fasting serum sugar level among patients on vitamin D3 supplements in group 1compared with those without vitamin D3 after 8 weeks of treatment.

After adjustment, the baseline means for treatment and control groups there was no significant changes in total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) after 8 weeks of treatment (*p*>0.05). There was a significant decrease in the mean of both SBP and DBP (*P*<0.05) in the treatment group compared to control group 2 after 8 weeks of treatment. Also, no significant difference was found in the mean atherogenic index between the two groups after 8 weeks of treatment (*p*>0.05).

Effect of Vitamin D3 Supplement on Biochemical Markers and Blood Pressure Reading According to Patients Demographic and Disease Characteristics

After adjustment of the baseline means for treatment and control groups according to the covariance analysis and taking the difference between variables mean of groups post-treatment and adjusted baseline,Table 4 show the overall effect of vitamin D3 supplement on different biochemical markers and blood pressure readings regarding age, gender, BMI and smoking status of subjects with cardiovascular risk.

Results revealed that vitamin D3 supplements produce a highly significant change (*p*<0.01) in serum Angiotensin II level in treatment group compare to control in both age groups (<60 years and ≥ 60 years) after 2 months of treatment. Also, the highly significant change was noticed according to gender, BMI (<30 and ≥ 30) kg/m^2, and smoking status.

The mean difference in endogenous vitamin D level was highly significant change (*p*<0.01) in the treatment group after vitamin D3 supplements compare to control in all studied variables as mentioned above.

Results also showed that vitamin D3 supplements did not produce any change (*p*>0.05) in serum blood sugar in the treatment group compared to control in all studied variables as well. Vitamin D3 supplements produced a significant change (*p*<0.05) in TC and LDL cholesterol of age group <60 years, particularly in females. All other studied

Table 2: Baseline demographic and disease characteristics of study groups

	Study groups		
Variable	Group 1	Group 2	P-value
Gender	n (%)	n (%)	-
Female	10 (50)	10 (50)	1.00 NS
Male	10 (50)	10 (50)	
Total	20 (100)	20 (100)	
Age (year)	55.35± 8.56	52.30± 9.17	0.284NS
BMI (kg/m²)	30.88 ± 4.7	28.83 ± 3.47	0.124NS
Smoking status	n (%)	n (%)	
Smoker	13 (65)	10 (50)	0.337NS
Non-smoker	7 (35)	10 (50)	
SGOT (IU/dl)	19.05±5.155	20.9±4.887	0.251 NS
SCr (mg/dl)	0.775±0.055	0.750±0.060	0.180 NS

Data presented as mean ± SD; Number of patients (n) and percentage (%); Two-sample *t*-test is used for statistical analysis of (age, BMI, SGOT, SCr); Chi-square test is used for statistical analysis of (gender and smoking status); NS is considered non-significant.

Table 3: Effect of Vitamin D3 supplement on biochemical markers and blood pressure reading

Variables	Groups	Pre-treatment mean	Post-treatment mean		P-value
			Mean	SER	
Ag II pg/ml	G1	169.79	102.411	8.994	0.005**
	G2		141.002	8.994	
D3 ng/ml	G1	11.26	36.827	2.724	<0.001**
	G2		14.753	2.724	
FBS mg/dl	G1	96.52	96.411	1.409	0.755 NS
	G2		97.039	1.409	
TC mg/ dl	G1	176.12	168.106	3.555	0.206 NS
	G2		174.644	3.555	
TG mg/dl	G1	129.95	124.604	6.550	0.786 NS
	G2		127.146	6.550	
HDL mg/dl	G1	41.60	40.489	1.044	0.475 NS
	G2		41.561	1.044	
LDL mg/dl	G1	107.42	109.033	5.648	0.114 NS
	G2		106.317	5.648	
VLDL mg/dl	G1	26.3	24.715	1.366	0.075NS
	G2		25.635	1.366	
SBP mmHg	G1	143.77	137.825	1.157	0.039*
	G2		141.375	1.157	
DBP mmHg	G1	90.05	86.083	0.812	0.013*
	G2		89.067	0.812	
Atherogenic Index	G1	0.441	0.434	0.028	0.526 NS
	G2		0.459	0.028	

Data presented as mean ± SER; NS: No significant differences (P>0.05), (*) Significant difference (P<0.05), (**) Highly Significant difference (P<0.01); Analysis of covariance (ANCOVA) test used to compare post-treatment between group 1 and group 2 patients, after adjustment of baseline reading of the same variables.

variables did not any change (p>0.05) in both treatment and control groups.

Moreover, results presented in Table 4a & 4b showed that vitamin D3 supplements produce a highly significant change (p<0.01) in SBP in the treatment group of obese females aged <60 years compared to control. Smoking seems to be an independent aff ecter. Meanwhile, the changes in DBP after vitamin D3 supplements produce a highly significant change (p <0.01) in the treatment group compared to control in all age group, both genders, obese and non-obese, and in different smoking status.

Finally, Table 4a & 4b showed that there was no significant change (p>0.05) in the atherogenic index after vitamin D3 supplements in the treatment group compared to control regarding age, gender, BMI, duration of disease, and smoking status after 8 weeks of treatment.

Table 4a: Mean differences between post-treatment and adjusted baseline groups mean of different biochemical markers and blood pressure reading according to ANCOVA analysis of subjects with cardiovascular risk

Variables	Groups	Age				Gender			
		<60YR		≥60YR		Male		Female	
		PO-PR	Sig	PO-PR	Sig	PO-PR	Sig	PO-PR	Sig
Ag II pg/ml	G1	-70.62	**	-101.14	**	-94.654	**	-75.221	**
	G2	-2.14		-18.42		-12.43		-10.029	
D3 ng/ml	G1	28.67	**	29.69	**	32.208	**	27.125	**
	G2	0.48		-0.76		0.152		-1.385	
FBS mg/dl	G1	0.18	NS	0.14	NS	2.234	NS	-1.782	NS
	G2	0.52		-0.04		2.466		-2.118	
TC mg/dl	G1	-10.14	*	-6.93	NS	2.66	NS	-5.164	NS
	G2	1.03		-2.97		-6.47		-0.296	
TG mg/dl	G1	-12.25	NS	-1.06	NS	-15.007	NS	1.332	NS
	G2	-3.02		0		-3.393		0.768	
HDL mg/dl	G1	0.88	NS	-1	NS	0.491	NS	-1.08	NS
	G2	-1.08		-1.1		-0.791		-0.92	
LDL mg/dl	G1	-10.025	*	-5.278	NS	5.223	NS	-14.28	*
	G2	4.165		-2.262		-5.043	NS	0.652	
VLDL mg/dl	G1	-2.396	NS	-0.212	NS	-3.001	NS	0.266	NS
	G2	-0.604		-0.048		0		0.154	
SBP mmHg	G1	-6.76	**	-8.03	NS	-8.434	NS	-6.298	**
	G2	0.26		-2.18		-2.766		0.798	
DBP mmHg	G1	-4.36	**	-5.46	**	-5.581	**	-4.197	*
	G2	0.46		-0.55		0.481		-0.603	
Atherogenic index	G1	-0.044	NS	0.031	NS	-0.043	NS	0.028	NS
	G2	0.015		0.02		0.004		0.034	

Table 4b: Mean differences between post-treatment and adjusted baseline groups mean of different biochemical markers and blood pressure reading according to ANCOVA analysis of subjects with cardiovascular risk

Variables	Groups	Weight status				Smoking			
		BMI <30		BMI ≥30		Yes		No	
		PO-PR	Sig	PO-PR	Sig	PO-PR	Sig	PO-PR	Sig
Ag II pg/ml	G1	-77.796	**	-97.388	**	-81.28	**	-89.4	**
	G2	3.353		-21.47		-0.9		-23.21	
D3 ng/ml	G1	31.472	**	29.41	**	28.2	**	32.08	**
	G2	-1.731		0.13		-0.63		-0.37	
FBS mg/dl	G1	1.629	NS	-1.62	NS	1.74	NS	-2.94	NS
	G2	-1.086		2		0.82		-1.1	
TC mg/dl	G1	-4.74	NS	-7.29	NS	-6.19	NS	-6.75	NS
	G2	-1.35		-5.33		-4.95		-1.28	
TG mg/dl	G1	-11.337	NS	-3.02	NS	-10.6	NS	-3.26	NS
	G2	-1.442		-2.35		0.88		-1.11	
HDL mg/dl	G1	-0.827	NS	0.36	NS	0.6	NS	-2.68	NS
	G2	0.134		-2.79		1.11		-2.34	
LDL mg/dl	G1	-0.786	NS	-7.653	NS	-5.58	NS	-3.77	NS
	G2	-2.259		-1.145		-5.06		-91.48	
VLDL mg/dl	G1	-2.267	NS	-0.604	NS	-2.12	NS	-0.66	NS
	G2	-0.288		-0.469		0.17		-0.22	
SBP mmHg	G1	-6.029	NS	-8.48	**	-6.88	*	-28.167	**
	G2	-0.397		-1.55		-2.36		0.313	
DBP mmHg	G1	-4.861	**	-4.69	**	-5.11	*	-4.25	**
	G2	-0.592		0.39		-2.06		1.88	
Atherogenic index	G1	0.014	NS	-0.03	NS	-0.02	NS	0.003	NS
	G2	0.024		0.013		0.007		0.025	

EL=estimated end line reading, BL= estimated baseline reading, NS=not significant (PV≥0.05), *= significant (PV<0.05), **= highly significant (PV<0.01)

Discussion

To the best search, confirmation of the low vitamin D status –CVD hypothesis was not fully addressed from randomized clinical trials (RCTs) of vitamin D supplementation, nevertheless, results from recent meta-analyses (Wang *et al.,* 2012), and cohort studies (Schöttker *et al.,* 2014, Ford *et al.,* 2014) reported an increased incidence of cardiovascular disease among individuals with low vitamin D status. Accordingly, in this study, tracking the change in the blood pressure readings with the restoration of low vitamin D status subjects with cardiovascular risk was the main target speculating further amelioration in the future cardiovascular events. All participants of the current study presented with different levels of cardiovascular risk, a total of 24 subjects are categorized as low cardiovascular risk who required conservative lifestyle management, 9subjects are categorized as moderate risk who required who required risk monitoring every 6-12 months, and 7 subjects are categorized as high risk who required who required risk monitoring every 3-6months according to the recommendations of ACC/AHA guideline on the assessment of cardiovascular risk. Moreover, an additional factor that contributes to increasing the level of cardiovascular events is the low vitamin D status that all participants selected accordingly.

Management of primary prevention includes; Lifestyle advice address management of dyslipidemia, Optimise use of antihypertensive agents, other cardiovascular protective therapies and achieve tight blood glucose control as appropriate. Also, in patients without evidence of arterial disease, treatment must be considered if the risk of CVD is >20% or more over ten years (Stewart *et al.,* 2017). Treatment will generally include, A lipid-lowering agent such as simvastatin 40 mg/day (or alternative) but no treatment targets are set. Personalized information on modifiable risk factors including physical activity, diet, alcohol intake, weight and tight control of diabetes, Advice to stop smoking, And advice and treatment to achieve blood pressure below 140 mmHg systolic and 90 mmHg diastolic (Stewart *et al.,* 2017).

The 2013 ACC/AHA guidelines recommend either a high-intensity or moderate-intensity statin regimen in patients who have an elevated ASCVD risk (≥ 7.5%) for primary prevention of cardiovascular disease (Goff *et al.,* 2014).

In the present study, all subjects with cardiovascular risk were aged between 40-70 years, and both genders were enrolled in the study. This was consistent with the age-standardization to the age groups 45–74 according to a number of deaths from IHD and CVD as reported from Western countries (Manson *et al.,* 2012, Müller-Nordhorn *et al.,* 2008). The subjects in this study were free from any disease that may alter vitamin D activation or metabolism mainly renal or liver disease confirmed by normal SGOT and Scr levels. Moreover, the potential effect of other confounders were adjusted at the baseline reading via the analysis of covariance.

The risk factors for cardiovascular diseases and the individual involvement of each risk factor differs between different communities or ethnic groups, the overall involvement of these risk factors is very consistent (Yusuf *et al.,* 2004). Some of these risk factors, for example; age, gender or family history, are immutable, meanwhile many important cardiovascular risk factors are modifiable such as prevention of hypertension, hyperlipidemia, diabetes (McPhee *et al.,* 2010), and obesity as a chief risk of atherosclerosis of the coronary arteries (Eckel, 1997). Body weight and BMI have a positive correlation with BP due to the accumulation of fat (Dua *et al.,* 2014). Body weight increased with age and then slightly decreased after 50 years, since in advanced age there is a decrease in muscle mass due to reduced amount of protein intake or decrease number and size of muscle fibres in degenerative diseases that associated with the advancing age (Verma *et al.,* 1987), meanwhile younger subjects have larger energy intake, fat-rich diet, and relatively less energy expenditure (Mungreiphy *et al.,* 2011). It is well known that high blood pressure increased with age (Suman and Kapoor, 1998). The prevalence of increased BP is higher among men than women since lower levels of BP among women may be attributable to a protective effect of estrogen. Besides, most women are non-smoker and non-drinker (Mendelsohn and Karas, 1999).

Additionally, premenopausal women have more lipoprotein lipase (LPL) activity. Meanwhile, men have a higher level of intraabdominal tissue this explains the greater prevalence of dyslipidemia and chronic heart disease (CHD) in men than in premenopausal women (Dua *et al.,* 2014).`

In the current study, the baseline endogenous vitamin D level of the selected subjects in both study groups had vitamin D deficiency (<20 ng/ml), and after treatment with a dose of100000 IU of vitamin D3 orally every 2 weeks in this study, highly significant increase in the mean total endogenous vitamin D in the treatment group when compared to pre-treatment level, which provided a good estimation of potential effect of study intervention in restoring the deficiency to the acceptable normal level (38.5 ±1.243ng/ml). One study suggested that the daily oral intake of vitamin D in sunlight deprived individuals should exceed 600 IU per day to secure a normal level of 25-hydroxy vitamin D (Glerup *et al.,* 2000). Only serum 25-OH vitamin D

level which is considered the best circulating biomarker of vitamin D metabolic status was measured which reflect the actual endogenous vitamin D level from dietary intake and sunlight exposure, in addition to the conversion of vitamin D from adipose tissue in the liver (Hollis, 2005). This biomarker (25-OH vitamin D) was more stable than the active form of vitamin D (1,25 (OH)2D), which has a short half-life (Jones, 2008).

In the present study, there was a significant decrease in the serum level of Angiotensin II ($p < 0.01$) in selected subjects after vitamin D3 supplementation compared to control patients on conventional therapy for hypertension. The role of vitamin D in modifying Angiotensin II level was elegantly studied by many researchers with considerable evidence shows that vitamin D deficiency is related to CVD risk factors. Experimentally,Li *et al.* (2002) reported that vitamin D as a potent negative endocrine regulator of the renin-angiotensin system (Li *et al.,* 2002), and that mice lacking the vitamin D receptor have elevated production of renin and angiotensin II, leading to hypertension, cardiac hypertrophy, and increased water intake (Li *et al.,* 2004). In human, a study by Forman *et al.* (2007) observed an association between measured vitamin D deficiency and increased risk for incident hypertension, and he concludes that vitamin D–hypertension association may be mediated by influencing both the renin-angiotensin system and vascular function (Forman *et al.,* 2007)

On the other hand a very recent cohort by Scragg *et al.* (2017), he reported that monthly vitamin D supplementation of 100,000 IU in adults with CVD and serum endogenous vitamin D level of less than 30 ng/ml for 2.5 – 4.2 years did not prevent CVD despite the mean 25 (OH)D concentration greater than 40ng/mL in the vitamin D group, and he declared that monthly doses of vitamin D are less effective in preventing disease than daily or weekly doses (Scragg *et al.,* 2017).

The effect of vitamin D3 supplementation on both angiotensin II level and endogenous vitamin D levels in the present study were significantly clear in all age group above 40 years old, male and female, obese and non-obese, smoker, and nonsmoker. Also, the metabolic parameters like FBS, TG, HDL-c, VLDL-c and atherogenic index did not show any difference considering patients demographics. Nevertheless, only TC and LDL-c significantly affected by vitamin D3 supplementation in obese females aged <60 years, hence all variables seem to have an indirect effect on the mechanism of vitamin D3 supplementation in cardiovascular pathology.

In this study, the SBP was highly improved by vitamin D3 supplementation particularly in obese females aged <60 years probably correlated with the TC and LDL-c levels. Meanwhile, the effect of supplementation was hard to be noticed on SBP in advanced age because of the age-related decline in cardiac performance. On the other hand, the DBP was significantly improved regardless of all variables of subjects at cardiovascular risk. This effect on DBP can be potentially due to the direct modulation of vitamin D3 supplementation on the peripheral vasculature as mentioned earlier.

CONCLUSION

Vitamin D deficiency may benefit the cardiovascular system (CVS) after restoring sufficient endogenous vitamin D level particularly through its effect on lowering BP values. Supplementation of vitamin D3 is inexpensive, and it could reduce the risk of developing cardiovascular events and could be assigned as a primary preventive therapy in those subjects at high cardiovascular risk. A larger population-based study using different vitamin D3 regimen is of great importance.

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Conflict of interest

The authors report no conflicts of interest in this work.

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