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Assessment of Vitamin D Circulating Level as A Risk Factor in Acute Coronary Syndrome Patients.

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ABSTRACT

Acute coronary syndrome (ACS) is a term that encompasses both unstable angina and myocardial infarction (MI). The pathophysiologic hall mark of ACS is atherogenesis and process that leads to plaque rupture. Worldwide, IHD was the second and third prominent cause of disability-adjusted life years lost in men and women. Risk assessment is done by using risk factors and risk markers. Vitamin D plays a typical hormonal role in all the major cardiovascular cell types including cardiomyocytes, arterial wall cells, and immune cells.This study represents an attempt to evaluate the role of this analyte as a risk factor for future ischemic coronary events. 160 subjects enrolled in this a case –control study; 80 patients diagnosed as ACS patients by expert physicians. An equivalent age and sex matched population without coronary disease with similar risk factors considered as a control group. Serum levels of Vitamin D were measured by using ELISA technique. There were significant differences in mean serum levels of Vitamin D, by patients and control, p value ≤ 0.05 . Independent sample t-test was used to compare means between two groups. Chi square (X^2) test and Fisher exact test were used to find the significance of the categorical variables. Circulating level of Vitamin D seems to be used as a risk factor for ACS; its sufficient level appeared to be protective against ACS events.

Keywords: ACS, Vitamin D, 25-OH Cholecalciferole, risk factor.

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INTRODUCTION

The term acute coronary syndrome (ACS) encompasses both unstable angina and myocardial infarction (MI). The pathophysiologic hall mark of ACS is atherogenesis and process that leads to plaque rupture [1].It has long been appreciated that ACS is the manifestation of sudden plaque rupture with subsequent occlusive or sub occlusive thrombus formation, leading to distal myocardial ischemia or myonecrosis [2]. Worldwide, ischaemic heart disease (IHD) was the second and third prominent cause of disability-adjusted life years lost in men and women [3]. Risk assessment is done by using risk factors and risk markers.

Vitamin D3 is a steroid pro-hormone, which is mostly derived from UVB-induced synthesis of 7-dehydrocholesterole in the skin. This endogenous synthesis is the chief source of vitamin D supply to the body and represents for approximately 90% of the vitamin D supply [4]. Vitamin D is likely one of the oldest hormones contributed in vital processes of potential relevance to cardiovascular disease, comprising cell proliferation and differentiation, apoptosis, oxidative stress, membrane transport, matrix homeostasis, and cell adhesion [5]. Vitamin D receptors have been present in all the major cardiovascular cell types including cardiomyocytes, arterial wall cells, and immune cells. Clinical studies have generally established an independent association between vitamin D deficiency and different manifestations of degenerative cardiovascular disease including vascular calcification [6].Vitamin D deficiency, as well as cardio-vascular disease(CVD) and associated risk factors are highly prevalent worldwide and frequently co-occur[7].A recent Workshop Consensus for Vitamin D Nutritional Guidelines estimated that approximately 50% and 60% of the elderly in North America and the rest of the world, respectively, donot have satisfactory vitamin D levels[7].Reasons for this widespread deficiency remain unclear but are likely related to factors such as urbanization, demographic shifts, decreased outdoor activity, air pollution and global dimming, as well asdecreases in the cutaneous production of vitamin D with age[8].

Objective:

Assessing the significance of Vitamin D serum level as a coronary artery disease (CAD) risk factor, andfinding out the most contributing serum level to the CAD risk.

MATERIALS AND METHODS

1. Subjects:

Between 1st December 2014and 31st March 2015,160 subjects were recruited in this study, 80 consecutive patients aged ≥ 40 years old, diagnosed by expert physicians to have ACS from coronary care units (CCU) in Merjan Medical City and Al-Hilla Teaching Hospital in Babylon Province, Hilla City, in addition to age and sex matched (80 subjects) without coronary disease with similar risk factors considered as control group. This study was performed in the laboratories of Biochemistry Department in collage of Medicine /University of Babylon.The overall mean age of patients with ACS and control were (60.28±12.02) and (58.21±11.61) years old, respectively. This study was matched for gender, the ratio of male: female was 2:1 for both sexes.

Exclusion criteria:

Patients having the following conditions were excluded: Diabetes mellitus, renal failure, patients on hormones, Vitamin D supplements or glucocorticoids, age<40 years, patients coming from outside the governorate, pregnant women, incapacity to provide informed consent, prior inclusion in the present study, patients with known history of thyroid, hepatic, or malignant disease, drug abusers and anemic patients.

Ethical Issues: included

- **a.** Approval of scientific committee of the Clinical Biochemistry Department in Babylon Medical College/ University of Babylon/ Iraq.
- **b.** Approval of Babylon Health Directorate/Ministry of Health & Information Center for Research & Development of Babylon Province.

c. The objectives and methodology were explained to all participants in the current study and their signed consent was gained.

Sample collection:

Five ml of blood were obtained from each subject by vein puncture in sitting or lying position, and then pushed slowly into disposable tubes containing separating gel. Blood in the gel containing tubes was allowed to clot at room temperature for 2 hours and then centrifuged at 1000 ×g for approximately 15 minutes then the supernatant were obtained and stored at -20˚C until analysis [8].

Materials:

Vitamin D ELISA Kit (Elabscience / China) Cat. No.: E-EL-0012.

Methods:

Vitamin D circulating level assayed by Elabscience (China) ELISA kit.Vitamin D Status was evaluated according to Endocrine Society Clinicl Practice Giudelines [9], as presented in table (1).

Table 1: Vitamin D status

Vit.D status	Serum 25-OH Vit.D level
Sufficient	**≥ 30 ng/ml**
Insufficient	**20-29 ng/ml**
Deficient	**< 20 ng/ml**

Statistical analysis:

The collected data were calculated and analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows version 20th version. Data were expressed as (mean ± SD). Independent sample t-test was used to compare means between two groups. Chi square (X^2) test and Fisher exact test were used to find the significance of the categorical variables. P values less than (0.05) were considered significant.

RESULTS

Differences of Patients with Acute Coronary Syndrome and control by Socio-Demographic Characteristics:

Table 2: Differences between patients and controls in residence, occupational status, educational status and family income

Variable	Study Groups		χ2	P-values	Odds Ratio (95% C.I.)
	Patients (%)	Control (%)			
Residence					
Urban area	43 (53.8)	44 (55.0)	0.025	0.874	0.951(0.510-1.772)
Rural area	37 (46.2)	36 (45.3)			
Occupational status					
Employed	10 (12.5)	19 (23.8)	3.411	0.065	2.180(0.942-5.046)
Non-Employed	70 (87.5)	61 (76.2)			
Educational status					
Illiterate **	35 (43.8)	28 (35.0)			
Primary school	23 (28.8)	20 (25.0)	0.044	0.834	1.087(0.499-2.368)
Secondary school	15 (18.8)	14 (17.5)	0.117	0.732	1.167(0.483-2.818)
Higher education	7 (8.6)	18 (22.5)	5.190	0.023*	3.214(1.177-8.777)
Family income					
low**	32 (40)	34 (42.5)			
low middle	20 (25)	18 (22.5)	0.165	0.683	0.847(0.38-1.8835)
high middle	18 (22.5)	20 (25)	0.012	0.912	1.0458(0.47-2.325)
high	10(12.5)	8(10)	0.282	0.594	0.752(0.264-2.146)

P<0.05 is significant, **reference group

The overall mean age of patients with ACS and control were (60.28±12.02) and (58.21±11.61) years old, respectively. There was no significant mean difference between the mean age of patients and control. This age matching helps to eliminate differences in parameters' results. This study was matched for gender, the ratio of male: female was 2:1 for both sexes. Table (2) shows that there was insignificant difference of patients with ACS and control by residence, occupational status, and family income ($p>0.05$), but there is significant difference between them in educational status, control were three times more likely to have higher education (OR=3.2).

Difference of patients and control in Vitamin D3 (25-cholicalciferol):

Table (3) demonstrated that there was a significant difference ($p<0.05$) in serum Vitamin D level between ACS patients and control groups. Table (4) demonstrated that vitamin D deficiency (<20 ng/ml) was recorded in 54% and insufficiency (20-29 ng/ml) in 43% of total patients of ACS whereas, only 3 % of the patients had adequate Vitamin D levels (≥ 30 ng/ml).

Table 3: Mean Differences of patients and control by Vitamin D serum level *p value ≤ 0.05 is significant

Variable	Group	N	Mean	S.D	P value
Vitamin D	Case	80	19.35	7.09	<0.001*
	control	80	54.11	28.31	

Table 4: Distribution of different Vit.D status between patients and control

Vitamin D	Study group		p-value	Odds ratio 95% C.I.
	Patients	Control		
Deficiency <20ng/ml**	43(53.8)	12(15.0)		
Insufficiency 20-29ng/ml	35(43.8)	13(16.3)	0.535	1.331 (0.540-3.282)
Sufficiency ≥30 ng/ml	2 (2.5)	55(68.8)	<0.001*	98.54 (20.93-463.91)

**p value ≤ 0.001 is significant, **reference group

DISCUSSION

The significant low vit.D level noticed in current study agreed with DeMetrio M *et al*[10], Bhougal BN *et al* [11], Lee JH *et al* [12], Welles CC *et al* [13] and Gunta SS *et al* [14] who reported that the possible correlation between Vitamin D insufficiency and cardiac events has been postulated to be associated with metabolic, pro-coagulant and inflammatory events that predispose to atherothrombosis. In addition, Vit.D insufficiency has been shown to be associated with endothelial dysfunction and subclinical atherosclerosis.

Below latitude of approximately 35°North, UVB radiation is sufficient for vitamin D3 synthesis all the year round [15] .The latitude of Al-Hilla city, Iraq,where this study was carried out,is 32.4°North. So we cannot suspect any problem with adequacy of sun exposure during the study period. Very low vitamin D levels have been reported from other Middle Eastern countries, particularly from Lebanon, Iran, Jordan and Turkey as reported by El-Tayeb IM *et al* [16].

This finding coincides with the fact that more than half of the studied group was urban (54%) who are mostly prone to the low vit.D level. Candidate reasons reported by Krugera MC [17] for decrement in vitamin D level in urban area include: air pollution/haze reduces the UVB needed to make Vitamin D, not going outdoors as much in rural area (feel less safe, availability of air conditioning, lack of gardens, working indoor along the day time). The reasons subsided behind this low level of Vitamin D may be attributed to the changes currently appeared in western design of the houses compared to the previous eastern design characterized by central

house yard full of sunlight, accompanied by the disappearance of gardens from homes as they converted to additional buildings either for commercial purposes, or residential ones. The bad unsecured situation in the country may lead to minimize the people's traffic in the parks for promenading. Air conditioning in house, office and cars to avoid the hot sun, accompanied by increased use of multi-media indoors - TV, DVD, and internet access represent recent contributing factors for significant hypovitaminosis D in societies as reported by El -Tayeb IM *et al* [16].

In addition to that even rural population who represents 46% of the patients in the present study tend to live urbanized life in their residence, and retrogression of agricultural lands by converting it to residential lands as reported in Annual Statistical Abstract 2011-2012 [18] had been increased resulted in lowering in the benefit effect of rural life on Vitamin D status as that reported by Goswami R *et al* [19]. Briefly the impact of civilization or modern life discussed earlier resulted in this high frequency of Vitamin D low level status.

Heshmat R *et al* [20] reported another reason which is represented by lack of vitamin D food fortification program which explain the high prevalence of vitamin D deficiency in Iran and other similar Middle East countries despite their sunny climate. Since endogenous synthesis is the main source of vitamin D supply to the body and accounts for approximately 90–95% of most people's vitamin D requirement and just <10% of the vitamin D supply derived from dietary sources, and as naturally occurring dietary sources of vitamin D are limited, and food fortification is optional, inconsistent, inadequate, or nonexistent so, all these reasons stand behind the situation of limited period of sun screen exposure, which results in low Vit.D serum level, as presented by Beveridge LA *et al* [21] and BassukSS*etal*[22].

The wide distribution of veiling between women (for religious reasons) may results in this significant low Vit.D level among women which agreed with AL-Turki HA *et al* [23]. Nevertheless, the role of veiling in vitamin D deficiency status in females in present study is doubtful, because an approximate rate of deficiency among men was observed (96%). In addition to backtrack in the number of working women and the subsequent homebound with aging may subside behind the fact that all of the females in this studied group had low Vitamin D serum level (37% insufficient and 63% deficient) as that presented by AL-Turki HA *et al* [23].

The elderly are particularly having insufficient level mainly because aging is associated with decreased concentrations of 7-dehydrocholesterol in the skin as reported by Makariou S *et al*[24] and may be due in part to reduced physical and outdoor activity . Obese individuals who represent 90% of the studied group (in term of WHR>1) are also at above-average risk, presumably because of decreased bioavailability of this fat-soluble vitamin resulted from sequestration in adipose tissue and reduced sun exposure in obese people (less exercising time). So with aging and rising obesity prevalence in society Vitamin D low serum level represents a real problem as reported by Danik JS *et al* [25].

Cigarettes smoking as a significant risk factor in the present study may contribute to the low Vitamin D level in men as reported by Kassi E *et al* [26]. Soft drinks is very cheap in our markets resulted in wide consumption by different age groups, leading to decrease Ca^{2+} level leading to depletion of Vitamin D stores to compensate this decrement which results in deficiency if not replenished, which agree with Lenny R *et al* [27] who reported that, who drank large amount of cola soft drink, had low levels of Vitamin D.

The distribution of different Vitamin D status among different risky groups:

The distribution of vit.D status in the studied group came in accordance with the study of lee JH *et al* [28] where they reported very high prevalence up to 75% as 25(OH)D deficient and 21% as insufficient, making a total of 96% of patients with abnormally low 25(OH)D levels who presented with coronary artery disease. The odds ratio of the sufficient level is (98.5) as demonstrated in table (4) meaning that the subject having sufficient level of Vitamin D about 99 times is likely to be protected from ACS events. This finding agreed with that reported in the review of P.E. Norman *et al* [29] ,Makariou S *et al*[24] and BassukSS*et al* [22] who assists the beneficial effect of protective Vitamin D supplements or correction of Vitamin D status in high risk groups, rather than using the controversial intervention of these supplements after CHD events had been occurred. Leading to the speculation that correction of vit.D status could play a role in the primary prevention of cardiovascular disease or what we call "prevention best than treatment", which also agreed with Nadir MA*et al* [30], and VacekJL*et al* [31]. A possible explanation to the protective effects of normal Vitamin D includes

decreased proliferation of cardiac muscle and increased contractility which increases cardiac function, decrease thrombogenicity and increase fibrinolysis as presented by Sebaaly A *et al* [32].

CONCLUSION

Inadequate Vitamin D serum level represents a risk factor for ACS events. Sufficient level of Vitamin D appeared to be protective against ACS events.

Recommendation

Vitamin D supplementation might reduce the risk of acute coronary syndrome in general population.

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