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Involvement of catecholamines in congestive heart failure, linked to lack of vitamin D and increased epidermal growth factor-receptor (EGF-R) production

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ABSTRACT

Objective: In order to evaluate the role of sympathoadrenomedullary system in the causative mechanism of the anginal attack, the hemodynamic changes in heart failure were observed in 50 patients with congestive heart failure (CHF), compared to 20 normal subjects, and 20 patients with essential hypertension (HTN), all of whom had similar lifestyles; the overall age range was 18-60 years. Hence, we compared vitamin D, parathyroid hormone (PTH), calcium, lipids, the acute phase reactant high-sensitivity C-reactive protein (hsCRP), fibrinogen, serotonin, norepinepherine (NE), and human epidermal growth factor receptor (EGF-R) between these groups. **Results:** This study confirms a strong association between catecholamines as well as EGF-R levels with PTH and low vitamin D levels, being related to hyperlipidemia and inflammation (hsCRP and fibrinogen) in CVD. **Conclusion:** This study indicates that increased levels of lipids, fibrinogen, PTH, proinflammatory marker(s), catecholamines, and EGF-R, as well as low vitamin D, contribute to the complex process of atherosclerosis in hypertensive patients that leads eventually to CHF.

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KEYWORDS

CHF;
25-hydroxy vitamin D;
Fibrinogen;
Depression;
Catecholamines;
EGF-R.

INTRODUCTION

Cardiovascular disease (CVD) being a leading cause of death, will reach epidemic proportions in developing countries by the year 2020[1]. Hypotheses concerning the pathogenesis of CVD have frequently included both hemostatic and inflammatory mechanisms. Fibrinogen levels are positively associated with risk of thrombosis as well as being an indicator of acute inflammatory response[2]. The epidermal growth factor receptor (EGF-R) is the prototypical member of the family of receptor tyrosine kinases, a wealth of evidence has established that all EGF-R family members are essential to normal cardiovascular development[3]. Early studies reported the prevalence of depression to be from 18% to 60% in patients with CHF[4]. Hence, it is important to develop means to more accurately predict CVD events as depression. Assessing for depression in the CHF patients requires more understanding of the risk factors for depression as well as their relation to risk information independent of high density lipoprotein (HDL) cholesterol, total cholesterol (TC)

and other typical cardiovascular risk factors. In addition, noncompliance because of cognitive impairment, depression can disrupt positive outcome. Several inflammatory markers including high sensitivity C-reactive protein (hsCRP) have been identified in atherosclerotic lesions[5]. This inflammatory process contributes to precipitate acute thrombotic complications[6]. Circulating levels of the pro-inflammatory cytokine(s), growth factor(s), and their soluble receptors(R), as EGF-R, may be associated with CHF severity[7]. Moreover, EGF-R is hypothesized to be involved in the compensatory mechanisms following cardiac stress, which needs to be explored in relation to the serum levels of vitamin D (25(OH)D) and catecholamine, namely; norepinephrine (NE) and serotonin, known to be increased in patients with CHF[8].

Therefore, this study aimed to evaluate circulating EGF-R concentrations and whether serum EGF-R is associated with serum levels of hsCRP, fibrinogen, 25(OH)D, PTH as well as seretonin and NE levels in CHF patients, and hence to explore the relationship between them. Since it is well known that subjects with CVD have multiple risk factors that potentiate each other, we limit our study to non-smoker, non-obese male individuals. This study is one of our group's studies on CV risk and CVD, to explore the mechanisms and consequences related to this disorder.

METHODS

Subjects

The studied groups included (n = 90) males, of which (n = 20) males served as healthy controls (group I). The control group was selected from subjects that attended the outpatient endocrine clinic at Ain Shams University Specialized Hospitals (ASUSH). None of the healthy controls were taking any medication or dietary supplements. Patients enrolled in the study were classified into the following groups: hypertensive subjects (group II) (n = 20), they were selected from patients attending the Cardiology Department ASUSH. Group III (n = 50) CHF patients admitted to the Intensive Care Unit-Cardiology Department ASUSH. They were diagnosed as CHF based on conventional criteria, i.e., typical clinical symptoms of exertional breathlessness plus echocardiographic evidence of impaired left ventricular (LV) systolic function (LV ejection fraction [EF] <45%).

The study was approved by the Committee on Medical Ethics of ASUSH. The study was carried out in accordance with the regulations and recommendations of the Declaration of Helsinki. All subjects gave their written informed consent prior to participation. A detailed medical history and drug treatment(s) were collected for all subjects. The following clinical information was obtained from all participants: current medication consumption, presence of macrovascular disease and family CV history. Exclusion criteria were unstable cardiac disease, such as decompensated heart failure or unstable angina pectoris, prior anthracycline-based chemotherapy, and serious uncontrolled concurrent disease, or concurrent malignancy, and liver disease. Significantly more CHF than HTN patients were taking angiotensin-converting enzyme inhibitors and/or angiotensin-receptor blockers (68% vs. 40%) and diuretics (63% vs. 14%); both $p < 0.05$. However, the proportion of patients taking other medications was similar in the HTN and CHF groups: antiplatelet agents, beta-blockers, statins, and antianginal agents (nitrates, calcium antagonists).

Body mass index (BMI) was calculated as an index of the weight in kilograms divided by the square of the height in meters.

Laboratory procedures

All subjects were advised to take no medication on the morning for blood sample collection. Plasma were separated, aliquoted first for the measurement of fasting blood glucose (FBG)[9] and lipids [total cholesterol (TC)[10] and triacylglycerol (TAG)[11]] by using standard enzymatic techniques. High-density lipoprotein (HDL) cholesterol was determined after precipitation of apolipoprotein B-containing lipoproteins[12]. Finally, low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald Formula (FF)[13]. The reference values for the lipid profile were according to established guidelines[14].

Fibrinogen levels were determined with the modified Clauss method (Technoclone GmbH, Vienna, Austria)[15]. Calcium was determined by reaction with methyl-thymol blue[16].

Plasma aliquots were kept frozen at -70°C for

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hsCRP, PTH, vitamin D, and EGF-R determinations using an enzyme-linked immunosorbent assay (ELISA kits) procedures. High-sensitivity C-reactive protein was determined using kits supplied from DRG International Inc (Mountainside, NJ). Immunoenzymetric assay was used for quantitative measurements of PTH from DIAsource ImmunoAssays S.A. (Belgium). Additionally, vitamin D was estimated as 25(OH)D in serum by EIA kit supplied by R&D Systems (Minneapolis, Minnesota, USA). However, EGF-R determination was done by kits from Invitrogen Corporation (Carlsbad, California, USA). All ELISA procedures were carried out according to the manufactures instructions. Finally, plasma levels of monoamines; serotonin and NE were estimated according to Pagel et al. (2000)[17].

DATA ANALYSIS

All statistical analyses were performed using SPSS version 17 software package. Data are presented as mean ± SD. To determine differences between groups, analysis of variance (ANOVA) followed by Bonferroni's post-hoc analysis was used for multiple comparisons between different groups. Correlations between different parameters were evaluated by Pearson's rank correlation (r). Significance was set at $P < 0.05$.

RESULTS

The clinical and demographic data of the participants are presented in TABLE 1. As indicated in TABLE 2, the mean ± SD of hsCRP, fibrinogen, EGF-R, Ca, PTH, and catecholamines levels were significantly higher in the CHF group compared with those obtained in the HTN group ($P < 0.000$). However, 25(OH)D showed a decreasing trend in this same direction. In CHF group (n= 50) (TABLE 3a), a significant negative association was found among fibrinogen and 25(OH)D. Also, a significant positive correlation was observed between catecholamines and TAG, hsCRP (serotonin), fibrinogen and EGF-R (NE). Moreover, EGF-R relation to hsCRP was positive and significant ($P < 0.05$).

Moreover, TABLE 3b, emphasizes the correlation coefficients in CVD patients (groups II, III) (n=70), where inflammation increased significantly when BP increased, which were dependent on the lipid profile significantly, and so did fibrinogen, PTH, catecholamines (serotonin and NE) and finally EGF-R increased in that direction significantly ($P = 0.0000$). However, 25(OH)D were negatively associated with them ($P = 0.0000$).

TABLE 1 : Clinical and laboratory characteristics of the studied groups; control subjects (group I), hypertensive subjects (HTN) (group II) and patients with congestive heart failure (CHF) (group III). Data are mean ± SD.

	Group I	Group II	Group III
n	20	20	50
Age (years)	49 ± 6	51 ± 6	52 ± 6
BMI (Kg/m^2)	20 ± 0.74	20.3 ± 0.7	20.2 ± 0.7
MAP (mmHg)	84.5 ± 2.6	102.5 ± 1.1 [a]	123 ± 3 [a, b]
FBG (mg/dL)	82.5 ± 6	84.25 ± 2.25	82.3 ± 5.6
TAG (mg/dL)	112.5 ± 4	173.45 ± 14 [a]	243 ± 17 [a, b]
TC (mg/dL)	167.5 ± 11	245 ± 7 [a]	314 ± 11 [a, b]
HDL-C (mg/dL)	45 ± 4	36 ± 1 [a]	23 ± 2 [a, b]
LDL-C (mg/dL)	100 ± 12	175 ± 8.5 [a]	243 ± 12 [a, b]
TC/HDL-C ratio	3.7 ± 0.4	6.8 ± 0.3 [a]	14 ± 1.4 [a, b]

NOTES: n = number of participants; BMI = body mass index; MAP = mean arterial pressure; FBG = fasting blood glucose; TAG = triacylglycerol; TC = total cholesterol; HDL-C = high-density lipoprotein–cholesterol; LDL-C = low-density lipoprotein–cholesterol.
a Significantly different from group I at P < 0.0000.
b Significantly different from group II at P < 0.0000.

TABLE 2 : Serum concentrations of the studied parameters in control subjects (group I), hypertensive subjects (HTN) (group II) and patients with congestive heart failure (CHF) (group III). Data are presented as mean ± SD.

	Group I	Group II	Group III
n	20	20	50
hsCRP (mg/L)	2.6 ± 0.46	5.2 ± 0.9 [a]	13 ± 1.2 [a, b]
Fibrinogen (mg/L)	207 ± 8	256 ± 13 [a]	416 ± 16 [a, b]
EGF-R (fmol/mL)	6.25 ± 1	9.25 ± 0.6 [a]	12 ± 0.7 [a, b]
Calcium (ng/mL)	10 ± 0.6	9.6 ± 0.6 [a]	12 ± 0.4 [a, b]
PTH (mg/L)	61 ± 2	141 ± 4 [a]	265.5 ± 12 [a, b]
Vitamin D (ng/mL)	45.6 ± 1	40.4 ± 1 [a]	36.4 ± 1 [a, b]
Serotonin (ng/mL)	100 ± 5.6	173 ± 12.2 [a]	229 ± 8.5 [a, b]
NE (pg/mL)	112 ± 11.4	466 ± 25 [a]	785 ± 79 [a, b]

NOTES: n = number of participants; hsCRP = high sensitivity C-reactive protein; EGF-R = epidermal growth factor receptor, PTH = parathyroid hormone; NE = noepinepherine.
a Significantly different from group I at P < 0.0000
b Significantly different from group II at P < 0.0000

TABLE 3a : Correlation coefficients (r) of different parameters in patients with congestive heart failure (CHF) (group III) (n=50). r = Pearson's rank correlation coefficients.

	Fibrinogen	EGF-R	Serotonin
TAG	---	---	0.281 *
hsCRP	---	0.28 *	0.341 **
25(OH)D	- 0.357- **	---	---
NE	0.343 **	0.373 ***	---

*** Correlation is significant at the 0.05 level.**
**** Correlation is significant at the 0.01 level**
***** Correlation is significant at the 0.0000 level**
--- Non significant correlation.

DISCUSSION

Evidence for the pathophysiologic role of both thrombotic and inflammatory influences on CVD continues to accumulate[2]. In this study, 25(OH)D deficiency is associated with increased CVD risk (fibrinogen) as well as depression, above and beyond established cardiovascular risk factors (BMI and lipids profile). The higher risk associated with 25(OH)D deficiency was particularly evident among individuals with high BP. Fibrinogen levels are known to be increased

TABLE 3b : Correlation coefficients (r) of different parameters in cardiovascular disease patients (groups II, III) (n=70). r = Pearson's rank correlation coefficients.

	hsCRP	Fibrinogen	PTH	25(OH)D	Serotonin	NE	EGF-R
MAP	0.899***	0.942 ***	0.947 ***	-0.845- ***	0.901***	0.848***	0.800***
Fibrinogen	0.923***			-0.895-***	0.913***	0.913***	0.878***
PTH	0.934***	0.960***			0.925***	0.888***	0.872***
25(OH)D	-0.844-***	-0.895-***			-0.815-***	-0.815-	-0.774-***
Ca	0.904***				0.861***		0.872***
Serotonin, NE	0.888***	0.913***	0.888***	-0.815-***			0.856***
hsCRP		0.923***	0.934***	-0.844-***	0.919***	0.888***	0.870 ***
HDL-C	-0.891-***	-0.930-***	-0.949***	0.855***	-0.882-***	-0.882-***	-0.843-***
TC, TAG, LDL-C	0.874***	0.903***	0.883***	-0.844-***	0.892***	0.892***	0.740***

***** Correlation is significant at the 0.0000 level** **--- Non significant correlation.**

by many non-genetic factors, e.g. advancing age, smoking, obesity, oral contraceptive use, and estrogen replacement therapy[2]. However, acquired metabolic conditions, e.g. obesity, insulin resistance and type 2 diabetes also increase fibrinogen levels, hence, we restricted our work on nonsmoker, non-obese, normoglycemic males with age and sex matched subjects. Fibrinogen has been identified as a major independent risk factor for CVD. Fibrinogen has also been associated with traditional cardiovascular risk factors, suggesting that elevation of fibrinogen may be a pathway by which these risk factors exert their effect. There are several mechanisms by which fibrinogen may increase cardiovascular risk[15]. First, it binds specifically to activated platelets via glycoprotein IIb/IIIa, contributing to platelet aggregation. Second, increased fibrinogen levels promote fibrin formation. Third, it is a major contributor to plasma viscosity. Finally, it is an acute-phase reactant that is increased in inflammatory states.

Serotonin receptors are involved in cardiac hypertrophy through the regulation of hypertrophic cytokines. Moreover, the generation of reactive oxygen species and inflammatory cytokines (hsCRP) through the activation of reduced nicotinamide-adenine dinucleotide phosphate [NAD(P)H] oxidase has been implicated in cardiac hypertrophy. associated with superoxide anion production[18]. Increased platelet aggregation, in response to inflammation, contributes to the development of atherosclerosis and increases the risk of CHF[19]. EGF-R is considered to play a role in cardiac (patho)physiology. Circulating levels of the pro-inflammatory cytokine(s) and growth factors are elevated in patients with CHF. Furthermore, these proteins are associated with heart failure severity and increased cardiomyocyte apoptosis in these patients[7]. The elevated serum EGF-R in CHF patients could suggest that EGF-R plays a role in cardiac pathophysiology, which may be relevant in the light of the mechanisms responsible for fibrinogen-related cardiac dysfunction. Hypothetically, loss of cardiomyocyteexpressed EGF-R may result in loss of functional myocytes leading to functional impairment and CHF[7]. In our CHF population, we observed an inverse association between se-

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rum EGF-R levels and both HDL-C and 25(OH)D.

One possible explanation for the increased serum EGF-R levels in CHF patients is increased shedding of cardiomyocyte membrane-expressed EGF-R. An alternative explanation for the increased serum EGF-R levels may be altered splicing of primary EGF-R transcripts. It can also be imagined that the EGF-R is produced as an epiphenomenon by other organs, or as a result of systemic responses, such as passive congestion, ischaemia or as an acute phase reactant(s), namely; hsCRP and fibrinogen.

When raised TAG coexists with an atherogenic cholesterol profile, the overall risk is enhanced[20]. Signaling in vascular cells involves primarily the mitogen activated protein kinase pathway with participation of the EGF-R[21]. The structural basis for the development of CHF occurs secondarily to post-myocardial infarction, hypertensive hypertrophy, or cardiomyopathy. Both cellular and extracellular factors are involved in the remodeling process and it is the combined action of these factors giving rise to changes in myocardial structure which eventually affects function[22]. In our study, many bioactive molecules such as cytokines/chemokines, bioactive peptides, and neurohormones which are operative in CHF likely contribute to the induction of EGF-R. For example, a specific cassette of transcription factors is likely induced with extracellular stimuli in the context of CHF which in turn induces EGF-R.

Fibrinogen was elevated in the CVD groups (II and III). It is quite possible that elevated levels of this protein over time contribute to the induction of EGF-R. Fibrinogen and Ca mediating platelet adhesion to injured endothelium or exposed subendothelium plays a critical role in the development of vascular disease[23]; whether this pertains to the development of CHF in humans' remains to be proven. Platelets also appear to be an integral part of the inflammatory process and may directly initiate an inflammatory response of the vessel wall[23].

The higher risk associated with 25(OH)D deficiency was particularly evident among individuals with high BP (correlated negatively). This is in accordance with the concept that CHF is well recognized to be associated with elevated inflammatory markers such as hsCRP. Cytokines may be involved in the pathogenesis of endothelial dysfunction by modulating the balance between production of growth factors receptors and vasoconstrictors[24], as we can see in our study. More importantly, there was a strong and independent correlation between hsCRP levels and catecholamines and 25(OH)D, suggesting that inflammatory mechanisms may be responsible for activating the depressive mechanisms in CHF.

Which Came First? Is depression associated with other conditions thought to be associated with 25(OH)D deficiency, such as heart disease and hypertension[25].

For example, there is a strong association between heart disease and depression, and countless theories to explain it. The obvious one—that heart disease would cause anyone to get depressed—is incorrect. You see, depression often precedes the heart disease, suggesting a third factor causes both[26]. Therefore, if heart disease is associated with depression then the possibilities are depression caused the heart disease, heart disease caused the depression, or an unknown factor(s), perhaps 25(OH)D deficiency, caused some portion of both the depression and the heart disease.

Does 25(OH)D Affect The Brain? Vitamin D rapidly increases the in-vitro genetic expression of tyrosine hydroxylase (the rate-limiting enzyme for the catecholamine biosynthesis) by threefold.

Levels of the catecholamine; serotonin and NE, has been identified to be increased in our patients with CVD and more in the CHF group. Norepinepherine is able to signal through both α- and β-adrenergic receptor systems. NE signaling through the Gαs-protein coupled β1 receptor results in the activation of adenylyl cyclase and increased cAMP levels. In addition, PKA can activate the MAPK pathway inducing other downstream responses[22]. By extension, activation of the MAPK pathway can induce transcription factors such as c-Jun, GATA-4, and NF-κB. Moreover, NE is able to signal through the α-adrenergic receptor system. The α1 receptor is coupled to a Gαq protein and results in the activation of PLC, IP3, DAG, and finally PKC.

CONCLUSION

In addition to systemic HTN and potentially vasculopathic medications, CHF patients are exposed to a number of atherogenic and vasculopathic factors including elevated TC/HDL-C ratio, TAG, fibrinogen, Ca, PTH and EGF-R levels, together with a reduced

25(OH)D and HDL-C level. It is likely that many of these factors contribute to the development of CHF with depression. The observation that many of these atherogenic-vasculopathic markers are already present in CHF patients raises the question of whether the treatable factors should be therapeutically approached (e.g., statin therapy, vitamin supplementation) after heart failure. Dyslipidemia and fibrinogen platelet activation, as surrogate markers of CV inflammation, are elevated in hypertensive patients and more in CHF patients. These patients present a high risk for CHF and need early aggressive intervention. Fibrinogen is of particular interest since it may act through two independent pathophysiologic mechanisms, i.e. thrombosis and inflammation. A better understanding of the genetic determinants of fibrinogen levels and how they influence the development of CVD and its complications will assist in fashioning better preventive and treatment strategies for this very serious public health problem. Serum EGF-R levels in our population of CHF patients were increased compared controls, which suggests that EGF-R plays a role in heart failure. The origin and underlying pathophysiological mechanisms for the higher circulating levels of EGF-R remain unclear and require further investigation. Evidence exists that depression is associated with CVD, HTN, and low bone mineral density, all illnesses thought to be caused, in part, by 25(OH)D deficiency. Finally, 25(OH)D has profound effects on the brain including the neurotransmitters involved in major depression.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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