Involvement of Depressive Catecholamines as Thrombosis Risk/Inflammatory Markers in Non-Smoker, Non-Obese Congestive Heart Failure, Linked to Increased Epidermal Growth Factor-Receptor (EGF-R) Production

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Abstract The EGF-R, also known as HER-1 or erbB-1 (EGF-R/HER-1/erbB-1), is a member of the human epithelial receptor tyrosine kinase family. sEGF-R is considered to play a role in cardiac (patho)physiology. We aimed to investigate whether soluble EGF-R is increased in congestive heart failure (CHF) patients and if related to disease severity. Soluble EGF-R, vitamin D, parathyroid hormone (PTH) was studied, and being evaluated in relation to Ca^{2+} , lipids, hsCRP, fibrinogen, serotonin, norepinephrine (NE). The study compared non-smoker, non-obese male CHF patients ($n = 50$) with age and gender-matched essential hypertension (HTN) patients ($n = 20$). Moreover, comparison with healthy control volunteers ($n = 20$) were employed. EGF-R/HER-1/erbB-1 was higher ($P = 0.013$) in 50 CHF male patients mean 12 ± 0.7 fmol/ml, than in 20 HTN, 9.25 ± 0.6 fmol/ml or in 20 controls, 6.25 ± 1 fmol/ml. Serum EGF-R levels correlated positively with hsCRP and NE, and were highest among CVD patients ($n = 70$) as well as negatively with vitamin D and HDL-C. EGF-R/HER-1/erbB-1 levels are increased in HTN and more in CHF patients. This study confirms a strong association between catecholamines as well as EGF-R/HER-1/erbB-1 levels with PTH and low vitamin D levels, being

related to hyperlipidemia and inflammation (hsCRP and fibrinogen) in CVD. Moreover, contributing to the complex process of the inflammatory component of atherosclerosis in hypertensive patients that leads eventually to CHF.

Keywords CHF · 25-hydroxy vitamin D · Fibrinogen · Depression · Catecholamines · EGF-R/HER-1/erbB-1

Introduction

Cardiovascular disease (CVD) being a leading cause of death, will reach epidemic proportions in developing countries by the year 2020 [1]. 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), also known as HER-1 or erbB-1, is the prototypical member of the family of human epithelial receptor tyrosine kinases. A wealth of evidence has established that all EGF-R/HER-1/erbB-1 family members are essential to normal CV development [3]. Currently, EGFR is considered as a convergence point in the complex signaling network and regulation of cellular functions, such as cell growth, differentiation, motility, survival and death. Thereby, it plays a crucial role in various pathophysiological processes, including atherosclerosis [4].

Associations between clinical psychological depression and CVD risk have become an increasingly common [5]. Hence, it is important to develop means to more accurately predict CVD events such as psychological depression. Several inflammatory markers, including high sensitivity C-reactive protein (hsCRP), have been identified in atherosclerotic lesions, contributing to acute thrombotic complications [6]. Circulating levels of the pro-inflammatory cytokine(s), growth factor(s) (GFs), and their soluble receptors (R), such as

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EGF-R/HER-1/erbB-1, 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, that are known to be increased in patients with CHF [8]. 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 typical CV risk factors.

We hypothesize that EGF-R/HER-1/erbB-1 is involved in CHF, reflected by altered sEGF-R/HER-1/erbB-1 concentrations. Hence, the aim of the present study was to evaluate circulating EGF-R/HER-1/erbB-1 concentrations, and whether serum EGF-R is associated with serum levels of hsCRP, fibrinogen, 25(OH)D, PTH as well as psychological depression in non-smoker, non-obese, male CHF patients, and moreover, to explore the relationship between them. This study is one of our groups' studies on CV risk and CVD in Egypt, to explore the mechanisms and consequences related to this disorder.

Subjects and Methods

Subjects

The study population comprised 90 males, of which twenty males served as healthy control volunteers (group I). 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, Ain Shams University Specialized Hospitals (ASUSH). Group III ($n = 50$) CHF patients admitted to the Cardiology Care Unit-Cardiology Department, ASUSH. Signs and symptoms of HF were classified according to the New York Heart Association (NYHA) functional class, with all CHF patients being class III. 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. Exclusion criteria were: unstable cardiac disease, 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 as well as diuretics. 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).

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

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)

Group, n	Group I, 20	Group II, 20	Group III, 50	P value
Age (years)	49 $\pm$ 6	51 $\pm$ 6	52 $\pm$ 6	NS
FBG (mg/dl)	20 $\pm$ 0.74	20.3 $\pm$ 0.7	20.2 $\pm$ 0.7	NS
BMI (Kg/m ²)	84.5 $\pm$ 2.6	102.5 $\pm$ 1.1 ^a	123 $\pm$ 3 ^{a, b}	NS
MAP (mmHg)	82.5 $\pm$ 6	84.25 $\pm$ 2.25	82.3 $\pm$ 5.6	<0.001
TAG (mg/dl)	112.5 $\pm$ 4	173.45 $\pm$ 14 ^a	243 $\pm$ 17 ^{a, b}	<0.001
TC (mg/dl)	167.5 $\pm$ 11	245 $\pm$ 7 ^a	314 $\pm$ 11 ^{a, b}	<0.001
HDL-C (mg/dl)	45 $\pm$ 4	36 $\pm$ 1 ^a	23 $\pm$ 2 ^{a, b}	<0.001
LDL-C (mg/dl)	100 $\pm$ 12	175 $\pm$ 8.5 ^a	243 $\pm$ 12 ^{a, b}	<0.001
TC/HDL-C ratio	3.7 $\pm$ 0.4	6.8 $\pm$ 0.3 ^a	14 $\pm$ 1.4 ^{a, b}	<0.001

Data are mean $\pm$ SD

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, b Significantly different from group I and II, respectively

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. After protocol approval, the study was conducted in the period from July 2009 to March 2010.

Body mass index (BMI) was calculated as an index of the weight in kilograms divided by the square of the height in meters. Detailed characteristics of the study groups are presented in Table 1.

Laboratory Procedures

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

Analytical Determinations

Fibrinogen levels were determined with the modified Clauss method (Technoclone GmbH, Vienna, Austria) [15]. Calcium was determined by reaction with methyl-thymol blue [16]. Another part of plasma/serum aliquots were kept frozen

at -70°C for hsCRP, PTH, vitamin D, and EGF-R determinations using ELISA kits procedures. hsCRP was determined using kits supplied from DRG International Inc (Mountainside, NJ). Immunoenzymetric assay was used for quantitative measurements of PTH from DAsource ImmunoAssays S.A. (Belgium). Additionally, vitamin D was estimated as 25(OH)D by EIA kit supplied by R&D Systems (Minneapolis, Minnesota, USA). However, sEGF-R/HER-1/erbB-1 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. [17].

Data Analysis

All statistical analyses were performed using SPSS version 17 software package. Values are given as the mean $\pm$ SD. Quantitative variables were compared between two groups using a Student's *t* test and ANOVA for more than two groups that are normally distributed. However, Mann-Whitney U-test was used for skewed distributed variables. In addition, for comparisons between more than two groups, a Kruskal-Wallis test was used. Correlations between variables were calculated using Pearson's correlation coefficient test. All *P* values were two-sided with *P* < 0.05 was considered statistically significant. Additionally, *P* < 0.001 was considered highly significant.

Results

As indicated in Table 2, the mean $\pm$ SD of hsCRP, fibrinogen, sEGF-R/HER-1/erbB-1, Ca²⁺, PTH, and

catecholamines levels (NE and serotonin) were significantly higher in the CHF group compared with those obtained in the HTN group (*P* < 0.001). However, 25(OH)D showed a decreasing trend in the same direction.

In CHF group (*n* = 50) (Fig. 1), 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 sEGF-R/HER-1/erbB-1 (NE). Moreover, sEGF-R/HER-1/erbB-1 relation to hsCRP was positive and significant (*P* < 0.05). Moreover, Table 3, 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 sEGF-R/HER-1/erbB-1 increased in that direction significantly (*P* < 0.001). However, 25(OH)D were negatively associated with them (*P* < 0.001) and positively with PTH. Moreover, neuro-hormones, namely NE and serotonin were correlated positively with sEGF-R/HER-1/erbB-1.

Discussion

In this study, 25(OH)D deficiency is associated with increased CVD risk (fibrinogen) as well as depression, above and beyond established CV risk factors (BMI and lipids profile). The higher risk associated with 25(OH)D deficiency was particularly evident among individuals with high BP. Acquired metabolic conditions, e.g. obesity, insulin resistance and type 2 diabetes also increase fibrinogen levels, hence, we restricted our work to non-smoker, non-obese, normoglycemic (data not shown) males with age- and sex-matched subjects.

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)

Group, <i>n</i>	Group I, 20	Group II, 20	Group III, 50	<i>P</i> value
hsCRP (mg/l)	2.6 $\pm$ 0.46	5.2 $\pm$ 0.9 ^a	13 $\pm$ 1.2 ^{a, b}	<0.001
Fibrinogen (mg/l)	207 $\pm$ 8	256 $\pm$ 13 ^a	416 $\pm$ 16 ^{a, b}	<0.001
EGF-R/HER-1/erbB-1 (fmol/ml)	6.25 $\pm$ 1	9.25 $\pm$ 0.6 ^a	12 $\pm$ 0.7 ^{a, b}	<0.001
Calcium (ng/ml)	10 $\pm$ 0.6	9.6 $\pm$ 0.6 ^a	12 $\pm$ 0.4 ^{a, b}	<0.001
PTH (mg/l)	61 $\pm$ 2	141 $\pm$ 4 ^a	265.5 $\pm$ 12 ^{a, b}	<0.001
Vitamin D (ng/ml)	45.6 $\pm$ 1	40.4 $\pm$ 1 ^a	36.4 $\pm$ 1 ^{a, b}	<0.001
Serotonin (ng/ml)	100 $\pm$ 5.6	173 $\pm$ 12.2 ^a	229 $\pm$ 8.5 ^{a, b}	<0.001
NE (pg/ml)	112 $\pm$ 11.4	466 $\pm$ 25 ^a	785 $\pm$ 79 ^{a, b}	<0.001

Data are presented as mean $\pm$ SD

n number of participants, *hsCRP* high sensitivity C-reactive protein, *EGF-R* epidermal growth factor receptor, *PTH* parathyroid hormone, *NE* neopinephrine

a, b Significantly different from group I and II, respectively

Fig. 1 Correlation coefficients (r) of different parameters in patients with congestive heart failure (CHF) (group III) ($n = 50$); NE (pg/ml) relation to fibrinogen (mg/l) (a), vitamin D (ng/ml) relation to fibrinogen (mg/l) (b), sEGF-R/HER-1/erbB-1 relation to NE (pg/ml) (c), and hsCRP (d), serotonin (ng/ml) relation to hsCRP (e), and TAG (mg/dl) (f). r = Pearson's rank correlation coefficients

Table 3 Correlation coefficients (r) of different parameters in cardiovascular disease patients (groups II, III) ($n = 70$)

	hsCRP	Fibrinogen	PTH	25(OH)D	Serotonin	NE	EGF-R/HER-1/erbB-1
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***

r = Pearson's rank correlation coefficients

*** Correlation is significant at the 0.001 level

— Non significant correlation

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]. Platelets serve major functions in three key aspects of atherosclerosis: atherogenesis, inflammation, and atherothrombosis [19] contributing to increasing CHF risk. Circulating levels of the pro-inflammatory cytokine(s) and GFs are elevated in patients with CHF. Elevated sEGF-R/HER-1/erbB-1 in CHF patients could suggest that sEGF-R/HER-1/erbB-1 plays pivotal role in cardiac pathophysiology, which may be relevant in the light of the mechanisms responsible for fibrinogen-related cardiac dysfunction. In our CHF population, we observed an inverse association between sEGF-R/HER-1/erbB-1 levels and both HDL-C and 25(OH)D. One possible explanation for the increased sEGF-R/HER-1/erbB-1 levels in CHF patients is increased shedding of membrane cardiomyocyte-expressed EGF-R. Moreover, an alternative explanation for the increased sEGF-R/HER-1/erbB-1 levels may be altered splicing of primary sEGF-R/HER-1/erbB-1 transcripts. It can also be imagined that the EGF-R/HER-1/erbB-1 is produced as an epiphenomenon by other organs, or as a result of systemic responses, such as passive congestion, ischemia or as an acute phase reactant(s), together with TAG, hsCRP, and fibrinogen.

Signaling in vascular cells involves primarily mitogen activated protein kinase pathway with participation of the EGF-R/HER-1/erbB-1 [20]. 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 [8]. In our study, many bioactive molecules such as cytokines/chemokines, bioactive peptides, and neurohormone(s), which are operative in CHF, likely, contribute to the induction of EGF-R/HER-1/erbB-1. This was the case in our study; EGF-R/HER-1/erbB-1 in the context of CHF was increased significantly.

Fibrinogen was elevated in the CVD groups (II and III). It is quite possible that an elevated level of this protein over time contributes to the induction of EGF-R/HER-1/erbB-1. Platelets appear to be an integral part of the inflammatory process and may directly initiate an inflammatory response of the vessel wall [19]. Vitamin D was correlated negatively to fibrinogen, neurohormones, explaining the depressive phase of CHF. Moreover, these later hormones increased

significantly associated with inflammation (hsCRP, fibrinogen, PTH, and EGF-R/HER-1/erbB-1).

In addition, only NYHA functional class III heart failure patients were enrolled. This indicates that patients' HF was well controlled. As a result, true congestion, which might have influenced sEGF-R/HER-1/erbB-1 and cytokine(s) level, was not observed.

The higher risk associated with 25(OH)D deficiency was particularly evident among individuals with high BP (negatively correlated). 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 productions of GFs receptors, as we can see in our study, and vasoconstrictors [21]. 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.

We, and others [22], tested whether depression is associated with other conditions, thought to be associated with 25(OH)D deficiency, such as heart disease and HTN. Countless theories to explain this, the obvious one—that heart disease would cause anyone to get depressed—is incorrect. Depression often precedes the heart disease, suggesting a third factor causes both [23].

Levels of the catecholamine; serotonin and NE, has been identified to be increased in our patients with CVD and more in the CHF group. NE signaling through the G α s-protein coupled β 1 receptor results in the activation of adenylyl cyclase and increased cAMP levels. In addition, PKA can activate the MAPK pathway inducing other downstream responses [8]. And if 25(OH)D affect brain, vitamin D rapidly increases in vitro genetic expression of tyrosine hydroxylase (the rate-limiting enzyme for the catecholamine biosynthesis) by threefold.

Conclusions

The observation that atherogenic-vasculopathic marker(s) are already present in CHF patients raises the question of whether the treatable factors should be therapeutically approached (e.g., statin therapy and/or vitamin supplementation) after CHF. sEGF-R/HER-1/erbB-1 levels in our population of CHF patients were increased compared to healthy controls, which suggests that sEGF-R/HER-1/erbB-1 plays pivotal role in CHF. Moreover, evidence exists that depression is associated with CVD, HTN, and low bone mineral density, all illnesses are thought to be caused, in part, by 25(OH)D deficiency.

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