Assessment of Serum Levels of Salusin α and Salusin β in Cardiovascular Disease Patients Undergoing Transcatheter Therapy

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Abstract

Cardiovascular disease (CVD) covers some disorders, such as the diseases of the cardiac muscles and the vascular system supplying the heart, brain and extra vital organs. CVD morbidity and mortality is mainly due to coronary heart disease and cerebro-vascular diseases. The aims of the present is to measure the levels of Salusin α, Salusin β, lipid profile, fasting blood sugar, insulin, insulin resistance and HOMA IR in the serums of cardiovascular patients who are subject to catheterization and to compare these levels with the ones of the healthy group and their correlation with Salusins. The results have made use of the appropriate statistical methods.

The results of the research have shown that there is a significant increase in the salusin-β in CVD patients compared with that of the healthy group but Salusin α ($p>0.001$) witnessed a significantly lower level. The present study has demonstrated that VLDL.C, HDL.C, TG.C and Salusin-β are in a significant correlation with Salusin α. Also, Age, BMI, HOMA-β, Insulin, HOMA-IR, FBG, CHO and LDL.C have no significant correlation with Salusin α. The results have shown that BMI and Salusin α levels are significantly correlated with salusin-β. In addition, Age, HOMA-β, Insulin, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, HDL.C and TG.C have no significant correlation with Salusin-β. BMI and HDL.C have shown a positive correlation with Salusin α. Moreover, Age, Insulin, HOMA-IR, HOMA-β, FBG ,CHO, LDL.C, VLDL.C and TG.C have shown a positive correlation with Salusin-β.

Key words: *Cardiovascular disease, Salusin α, Salusin β.*

Introduction

Cardiovascular disease (CVD) covers disorders like the cardiac muscles and the vascular system supplying the heart, brain and extra vital organs (1). CVD morbidity and mortality is mainly due to coronary heart disease and cerebro-vascular diseases (2). CVD is as a rule connected with atherosclerosis; an inflammatory disease characterized via the accumulation of lipids and fibrous elements in relation to medium arteries (3).

Salusins are taken as a category of bioactive peptides discovered through bioinformatics analyses of a complete length CDNA library. Recently, two associated peptides of 28 and 20 amino acids are renowned and characterized; they specified Salusin-α and Salusin-β. These peptides are believed to be biosynthesized of pre- prosalusin; an alternative-splicing production of the torsion dystonia–related gene (TOR2A), subsequent to frame shift reading and digestion at dibasic amino acids [(4)].

Salusins are synthesized ubiquitously in human tissues, counting the vasculature, central nervous system and the kidney. Salusin-α is present inside human plasma and urine[(5)]. Salusin-β quickly induces hypotension, bradycardia, and cardiac dysfunction during a cholinergic mechanism [(6)]. Salusin- β also stimulates human macrophage foam cell formation [(7)], proliferation of vascular smooth muscle cells and fibroblasts [(4)] and cardiomyocyte growth and anti-apoptosis [(8;9)].

Material and Method

A case control study is designed for a total of 60 subjects (44 males and 16 females, aged between 35 to

65 years) who consecutively registered in this study. The subjects are 30 Iraqi patients with cardio vascular disease (CVD). They have participated in the current study (8 female and 22 males). These patients are registered as CVD patients in the "open heart Unit" at "AL-Sader Teaching Hospital" in Najaf, Iraq. The patients' serum in the pre - and post-cardiac catheterization is collected and compared with that of the control group. Thirty healthy adults are selected as the control group. The range of their ages is analogous to that of the patients (35-65) years. Subjects who suffer from the apparent diabetes mellitus, acute infections, chronic liver diseases, renal disorders, cancers and patients with surgical procedures in the last 3 months, and nonsmoker are all excluded.

Five milliliters of venous blood after 12 hours fasting are drawn from the CVD patients and the healthy group during (8:30-10 A.M) from antecubital venipuncture using G 23 needles.

Hypertension is diagnosed as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg. The BMI is calculated as the ratio of weight (Kg) to height squared (m^2), by unit kg/m^2. Fasting analysis of serum glucose, lipid profile (CHO, TG.C, LDLC, and HDL.C) levels are measured by colorimetric method for the quantitative *in vitro* diagnostic measurement using kit (BIOLABO (France)). The Salusin α and Salusin-β are also measured using the Competitive-ELISA principle (Elabscince (USA)).The concentrations of Insulin serum are determined by ELISA kits (Calbiotech (USA)). Insulin resistance index (Homeostatic model assessment-insulin resistance,HOMA-IR) is estimated as follows: HOMA- IR = [glucose (in mg/dL) * insulin (μU/ml)] / 405.HOMA -β =360× Insulin /(Glucose-63) % [10].

Bio-statistical Analysis

The results are subjected to statistical analysis and are analyzed using Microsoft Excel 2013 and SPSS-20 (statistical package for social science-version 20). The results are expressed as numbers and as mean ± SD (Standard deviation). The significance of difference is assessed using paired t-test for two dependent means. The correlation of parameters is determined using Pearson's correlation coefficient, taking $p \leq 0.05$ as the lowest limit of significance [11].

The one-way ANOVA (Analysis of variance) and Fishers Least Significant Difference (LSD) are applied to compare the differences among the studied groups.

Results and Discussion

Clinical characteristics of the Studied Groups

The patients with cardiovascular diseases have shown a significantly higher level of fasting blood sugar (by definition, ($p<0.001$), higher level of insulin($p<0.001$), respectively), higher level of HOMA IR($p<0.001$), Lipid profile ($p<0.001$) and Salusin-β ($p<0.001$) in CVD patients (Group2), compared with the healthy group (Group1). Additionally, lower Salusinα and HOMA-β ($p>0.001$) are also seen, table (1).

Table (1) shows significantly higher levels in the Insulin, FBG and HOMA IR ($p<0.001$), except HOMA-β which records a non significant level in pre-catheterization (Group2), compared with post-catheterization (Group3). No significant difference is registered regarding salusin-β and insulin ($p>0.001$)

But there is a significant increase in FBG, HOMA IR, HOMA-β,Lipid profile, Salusinα, when the control (Group1) is compared with post-Catheterization (Group3).

The demographics and laboratory data of the Salusin α and Salusin β groups are summarised in tables (2) and (3). The levels of VLDL.C, HDL.C, TG.C and Salusin β are significantly higher with Salusin α for all ($p<0.05$)($p=0.036$) ($p=0.022$) ($p=0.035$), ($p=0.048$), respectively.

There is no significance correlation of Age, BMI, HOMA-β, Insulin, HOMA-IR, FBG, CHO and LDL with Salusin α ($p>0.001$), as in table (2).

BMI and Salusin α levels are significantly elevated with Salusin-β ($p<0.05$) ($p=0.033$) ($p= 0.048$), respectively. Moreover, Age, HOMA-β, Insulin, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, HDL.C and TG.C have

no significant correlation with Salusin-β($p>0.001$), as in table (3).

This study has found that HDL.C is positively correlated with Salusin α. In addition, Age, BMI, Insulin, HOMA-β, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, TG.C and Salusin-β showed a negative correlation with Salusin α, as in table (2).

Furthermore, HDL.C and Salusin α showed a negative correlation with salusin-β. Also, Age, BMI, Insulin, HOMA-IR, HOMA-β, FBG ,CHO, LDL.C, VLDL.C and TG.C showed a positive correlation with Salusin-β., as seen in table (3).

CVD is as a rule connected with atherosclerosis (3). Atherosclerosis is a chronic vascular disease, in which the arteries are thicken and lose their flexibility as a result of cholesterol sedimentation in the artery wall. In the early stages of the disease, cholesterol accumulates in arterial macrophages, alter them to lipid-loaded bubbles cells. Wide atherosclerosis narrows the artery lumen to reduce blood flow, and the can enhance the complete blockage of the artery (12; 13). Atherosclerosis occurs due to a variety of reasons, the most significant of which is the deposition of large amounts of cholesterol and calcium in the blood. It may happen because of obesity overcharged as a result of absence of exercise. Additionally, the increased blood pressure raises the risk of developing atherosclerosis (14; 15).

Table (1) Biochemical characteristics of CVD patients and healthy as control group

Parameters	Control group1 Mean±SD	Pre-catheter group2 Mean±SD	Post-cathetergroup3 Mean±SD	P value
Age	28.01±1.34	29.11± 1.27	29.11± 1.27	a) NS b) NS c) NS
BMI	26.32 ± 3.329	31.46 ±5.010	31.46 ±5.010	a) 0.001 b) 0.001 c) 0.001
SBP (mmHg)	122.6±1	145±2	139.5±2	a) <0.0001 b) <0.0001 c) NS
DBP (mmHg)	70.7±2	76.3±2	71.2±2	a) NS b) NS c) NS
FBG (mg/dl)	99.29 ± 8.52	166.62±35.24	143.87±36.17	a) 0.000** b) 0.001 c)0.019
Insulin(µlu/ ml)	10.67±3.30	19.43± 10.85	8.94± 4.05	a)0.000** b) NS c) 0.000**
HOMA IR	2.59 ±0.82	8.03 ± 4.85	3.33 ± 2.21	a)0.000** b) 0.001 c) 0.000**
HOMA-β	111.42±43.88	145.58±369.51	45.42±24.85	a)0.617NS b) 0.001 c) 0.151 NS
HDL.C	55.61±6.33	37.43 ±8.41	39.07±6.97	a)0.000** b) 0.001 c) 0.419 NS
VLDL.C	20.23±3.31	51.48±16.86	39.79±13.28	a)0.000** b) 0.001 c) 0.005**
LDL.C	86.00±14.95	185.48±45.20	165.93±45.37	a)0.000** b) 0.001 c) 0.106 NS

Cont... **Table (1) Biochemical characteristics of CVD patients and healthy as control group**

CHO	161.85±15.41	274.54±43.68	244.80±42.91	a)0.000** b) 0.001 c) 0.011
TG.C	101.28±16.99	258.02±84.39	198.95±65.81	a)0.000** b)0.001 c) 0.004**
Salusinα	11.00± 1.68	10.59± 1.35	13.10 ±1.85	a) 0.303 NS b) NS c) 0.000**
Salusinβ	11.87± 1.38	14.63± 1.10	10.86 ± 2.21	a) 0.03 b) 0.001 c) 0.001

a) Significant difference between values in Group(1) and Group (2), **b)** Significant difference between values in Group (3) and Group (1), **c)** Significant difference between values in Group (3) and Group (2), **=significant differences at 1%, NS =non-significant at the 0.05 level, FBG: fasting blood glucose, HOMA-IR: Homoeostasis model assessment-insulin resistance.Salusin-α has a mild hypotensive effect (4) and suppresses human foam cell formation via the down-regulation of acyl-CoA : cholesterol acyltransferase-1 (ACAT-1), which stores cholesterol ester changed from free cholesterol in macrophages. In previous studies, Serum salusin-α levels are significantly decreased in acute coronary syndrome (ACS) patients as compared with healthy people and are less in accordance with the severity of coronary atherosclerotic lesions amongst ACS patients. In coronary atherosclerotic lesions of ACS patients, the level of expression of Salusin-α is lower than that of Salusin- β (7).

Salusins essentially affect the cardiovascular system (6;7). Salusin-β is the most hypotensive peptide, its infusion is rapid and profoundly decreases blood pressure and heart rate. Moreover, it is demonstrated to cause cardiac dysfunction through a cholinergic mechanism in rats (6;8). It may have significant roles in myocardial growth and hypertrophy. Salusin-α and salusin-β show converse actions on atherosclerosis due to their opposite regulatory effects on acyl-coenzyme A: cholesterol acyltransferases-1 (ACAT-1). Both of the formation of macrophage foam cells and the enlargement of atherosclerosis are suppressed via Salusin-α. Serum Salusin-α levels are also reported to be significantly lower in patients with coronary artery disease and hypertensive patients where their Salusin-α level is inversely associated with carotid atherosclerosis (7;16).

Table(2) The relevance of Salusin α with concentrations of biochemical parameters in the patients group

Variables	r	P
Age	-0.000	1.000
BMI	-0.304	0.109
FBG	-0.164	0.394
Insulin(μIU/ml)	-0.179	0.353
HOMA-IR	-0.227	0.237
HOMA-β	-0.077	0.693
CHO	-0.194	0.314
LDL.C	-0.119	0.538
VLDL.C	-0.392*	0.036
HDL.C	0.425	0.022
TG.C	-0.394*	0.035
Salusin β(pg/ml)	0.371*	0.048

P- Value ≤ 0.05 = significant, r : Pearson correlation

Table(3) The relevance of Salusin β with concentrations of biochemical parameters in the patients group

Variables	r	P
Age	0.182	0.345
BMI	0.397*	0.033
FBG	0.122	0.527
Insulin(μIU/ml)	0.127	0.511
HOMA-IR	0.102	0.597
HOMA-β	0.225	0.240
CHO	0.038	0.844
LDL.C	0.118	0.542
VLDL.C	0.150	0.438
HDL.C	-0.125	0.517
TG.C	0.149	0.441
Salusin α (pg/ml)	-0.371*	0.048

P- Value ≤ 0.05 = significant, r : Pearson correlation

Angiotensin II is associated with the genesis of arterial hypertension and cardiovascular remodeling (17;18). Renin-angiotensin system intervention in hypertensive patients has shown lower morbidity and mortality (19; 20). Salusin-β gene silence has normalized the increased circulating Ang II levels in addition to the local An II contents in both myocardium and mesenteric artery in spontaneously hypertensive rats (SHR). Additionally, the up-regulation of AT receptors within myocardium and mesenteric artery in SHR (4) are inhibited through the knockdown of Salusin-β.

The inhibitory effect of Salusin-β on the activation of angiotensin system may partially contribute to the attenuation of hypertension and cardiovascular remodeling. It is well known that increased oxidative stress is associated with endothelial dysfunction, apoptosis, hypertrophy, inflammation, fibrosis and cell migration relative to vascular remodeling of hypertension (21;22).

Conclusion

In conclusion, our results suggest that Salusin-α and Salusin-β prove contrasting effects on atherosclerosis and that Salusin-α and Salusin-β possess anti-atherogenesis and proatherogenesis, respectively.

The current study has concluded that in patients with CVD, Salusin β levels have recorded a significant increase in the serum of CVD.

Further, Lipid Profile changes are directed by the age of patients in CVD.

Salusinα increase in serum of post catheterization and Salusin β decrease in serum of post catheterization.

In order to improve diagnosis and treatment of CAD, the research community needs to understood how the immune response is analogous and how it differs in men and women with atherosclerosis.

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