Assessment of Serum Afamin and Preptin Levels as a Potential Diagnosis Markers for Cardiovascular Patients Undergoing Catheterization

Mohammed Saeed Salman Hasan[1], Muthana Saleh Mashkur[2], Seyed Omid Ranaei Siadat[3], Hanaa Addai Ali[4]

[1]B.Sc. Chemistry, [2]Assistant Proffesor, 1Department of Chemistry, College of Science, University of Kufa, Iraq, [3]Proffesor, Shahid Beheshti University Protein Research Center Tehran, Iran, [4]Proffesor, 1Department of Chemistry, College of Science, University of Kufa, Iraq

Abstract

The Cardiovascular disease (CVD) is a main cause of worldwide morbidity and mortality overwhelms any of the circulatory system disease. Therefore, The aims of presented study were to assess whether an Afamin, and Preptin levels are associated with Cardiovascular diseases; to find if there is an association between Afamin and preptin levels with insulin resistance in progressive of patients with cardiovascular diseases before and after treatment by catheterization.

A case-control study,comprised of 60 patients diagnosed as cardiovascular disease(30 male,30 female), their ages ranged between 35-65 years old, and were matched with patients age and number of male and female of 60 healthy control. The estimation of the levels of biochemical parameters in the patients and control groups revealed a significant elevations ($P < 0.01$) of the of Preptin (85.73±41.97 vs 41.58±23.50 pg/mL), Insulin (17.53±10.94 vs 6.89±4.71 µIU/mL), HOMA- IR (8.03 ± 4.85vs 2.39 ±0.82) in patients before treatment by catheterization than the control group. But, they were significantly lower($P<0.01$) in Afamin level (1.59±0.36 vs 1.95±0.26) and HOMA-β (111.42±43.88 vs 145.58±36.51 pg/mL), in patients before catheterization, when compared with healthy group. Furthermore, the result showed, significant decreases ($P<0.01$) of Preptin (1.94±0.33 pg/mL) Insulin(9.99±3.62 µIU/mL), HOMA-IR (3.03 ± 1.21). Also, were shown a significant increased ($P<0.01$) in level of Afamin in patients groups to (1.94±0.33 pg/mL) after treatment by catheterization. The result demonstrated that Afamin levels were a significant negative correlation with preptin and HOMA-IR. On the other side the preptin levels revealed a significant positively connected with BMI, Insulin and HOMA-IR.

The conclusions of the current study for the first time revealed that circulating Afamin and Preptin levels are strongly involved in the progress of cardiovascular diseases and could independently predict pathogenesis improvement of the of cardiovascular disease. They were associated with atherosclerosis disease that was considered one of the most important leading causes of Cardiovascular disease.

Keywords: *Cardiovascular disease, Catheterization, Afamin, Preptin, Insulin resistance.*

Introduction

Cardiovascular diseases (CVDs) involve the cardiovascular system: heart, blood vessels, and the circulatory. CVDs remain the biggest cause of deaths worldwide [1].Cardiovascular diseases is sometimes called “heart disease”, in medical terms, they are not precisely the same thing. Heart disease is a universal item for states affecting the configuration of the heart and the way it works[2].

All heart diseases consider cardiovascular diseases. Anyway, not all cardiovascular diseases consider heart diseases. For instance stroke that affects blood vessels of the brain, but not the heart himself[3].

One of the major risk factors of cardiovascular diseases is arteriosclerosis; it builds up when the arteries that supply the blood to the heart become partially or wholly blocked. This is usually caused by fatty deposits built up inside the arteries [4].

Human Afamin is a serum glycoprotein and was presentedto be anexact binding protein for vitamin E possibly responsible for vitamin E that moves in body fluids [5].

Preptin is a new hormone that is derived from proinsulin -like growth factor I and is statedto play role in mineral metabolism. It is produced in pancreatic β-cells, and is co-secreted with insulin by the cells[6]. Preptin consider a 34-amino acid peptide hormone consecrated from the β cells of pancreas sideways with insulin, amylin, and pancreastatin[7]. Preptin, a peptide first explored in 2001 . Males have less preptin levels than females [8]. Preptin is thoughtto be a physiological insulin emissionenhancer induced by glucose [9].

Materials and Method

A-case control study design,In total,of 60 subjects aged 35–65 years. The study case group contained30 patients that have CVD(21 males,9 females) and they are admitted to hospital. All diagnosed in the "open heart Unit" at "AL-Sader Teaching Hospital" in Najaf Province-Iraq. During the period from November 2018 to March 2019. And 30 healthy volunteers age and gender matched the patientsas the controlgroup. A detailed interview addressing personal history,blood pressure family history, demographic information and laboratory examination was performed.All the patients underwent cardiac catheterization and Serum collection for these patients in the pre - and post- cardiac catheterization was done.

Patients that havehepatic disease, strokes,renal disease, any acute or chronic inflammatory illness, pregnancy and lactating mothers, alcoholics, cerebrovascular accidents, rheumatoid arthritis, autoimmune disease, patients of juvenile and type1 diabetes mellitus were expelledfrom the study.

All members have given written approvaland this protocol was permitted by the moral and human research committee.

Collection of Specimens and Biochemical Analysis: Blood sample was drawn from fasting venous from all the subjects, 5ml of blood after 12 hours fasting were drawn from CVD patients and healthy group among 8:30- 10A.M .

Hypertension was diagnosed as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mm/Hg.The BMI was measured as the relationof weight (Kg) to height squared (m^2), by unit kg/ m^2, fasting analysis of serum glucose, lipid profile (TC, TG. LDL.c, and HDL.c) levels were calculated by colorimetric method for the quantitative *in vitro* diagnostic measurement using kit (BIOLABO (France)).

The Afamin and Preptin were using the Competitive ELISA principle (Elabscince (USA)). The serum Insulinconcentration were definedby ELISA kits (Calbiotech (USA)).Insulin-resistance index (Homeostatic model assessment-insulin resistance (HOMA-IR) was estimated as follows: HOMA-IR= [glucose (mg/dL)Xinsulin(μU/ml)]/405.

HOMA-β = 360×Insulin/(Glucose-63) % [10].

Biostatistical Analysis: The results were subjected to statistical analysis and analyzed using computer facility of Microsoft Excel 2013 and SPSS-20 (statistical package for social science-version 20).The results were presented as numbers, and mean ±SD (Standard deviation). Significance of difference was assessed using paired t-test for two dependent means. The one-way ANOVA (Analysis of variance to compare the differences among the studied groups. The correlation of parameters was determined using Pearson's correlation coefficient, taking $p \leq 0.05$ lowest limit of significance[11].

Results and Discussion

The clinical feature of the CVD patients and controls are shown in Table (1)which consists of the data of both patients before and after catheterization and the control group. In the current study, there was no significant in age .While, BMI, SBP, and DSP showed a significant increase in patients compared control group. The present result has found a higher significance in the fasting blood glucose (p=0.01), Insulin (p=0.01) HOMA- β(p=0.01) and HOMA IR (p=0.01) in all these groups, except Insulin (p=0.459) compared between the Control and Patients(Post- catheterization) also, HOMA-IR value (p=0.438)compared between values of Patients(Post- catheterization)and the Control group. The total serum of cholesterol, triglycerides, LDL.c levels were increased significantly, while, decreased

levels of HDL.c($p<0.001$) was seenin CVD patients when matched to control group.

As presented in table (2) revealed univariate analysis of Afamin with biochemical parameters that the all parameters have a positive correlation with Afamin, except theAge, BMI,FBG, HOMA-IR, TG, TC, LDL.c VLDL.c, Preptin, shown a negative correlation. There is a significance negative correlation between Afamin with the levels of HOMA-IR, TG, LDL.c, VLDL.c and Preptin levels.

In table (3) shown the Preptin levels have a significant positive correlation with BMI, insulin, HOMA-IR. While, a significant negative correlation with Afamin levels.

Cardiovascular diseases (CVD) and artery diseases (CAD) are the leading causes of morbidity and mortality in the developed countries and are emerging as an epidemics in the developing countries [12].

Coronary Heart Disease (CHD) is the most common reason of death from CVD. The risk factors such as smoking, diabetes mellitus, hypertension, high dietary fat intake, body mass index,lack of physical exercise, besides the traditional lipid panel which have been recorded to be risk factor by itself for the development of CVD [11].

Insulin performed like an endothelial reliant vasodilator in physiological levels, but Insulin resistance or hyperinsulinemia cause the loss of NO bioactivity in the wall of the vessel and thereby to endothelial dysfunction[13] .Compensatory hyperinsulinaemia happens when pancreatic β cell secretion risesto preserve normal blood glucose levels in the setting of peripheral Insulin resistance in adipose tissue andmuscle[14]. CVD is as a rule connected with atherosclerosis, Atherosclerosis is a chronic vascular disease, which the arteries experience lose flexibility and thicken as a result of cholesterol sedimentation in the wall ofartery.

In the first stages of the disease cholesterol gathers within arterial macrophages, changethem to bubbles cells that arelipid-loaded.[4] And atherosclerosis it may happen .

Table (1) Clinical parameters compared between patients before and after catheterization and control group because of obesity overcharged as a result of absence of exercise, and increased blood pressure raise the risk of developing atherosclerosis [15].

Parameters	Control Mean± SD No.30	Patients Pre-cath. Mean±SD	Patients Post-cath. Mean±SD	P- value
Age	55.265±7.5	53.61±9.2	53.61±9.2	a) NS b) NS
BMI	26.965±4.9	36.665±5.2	36.665±5.2	a) <0.01 ** b) <0.01 **
SBP (mm/Hg)	128.5±3.7	144.5±3.8	139.5±4.4	a) <0.01 ** b) <0.01** c)<0.01**
DBP (mm/Hg)	75.5±3.2	93±3.5	93±4.1	a) <0.01** b) <0.01** c) <0.01**
FBG (mg/dl)	97.78±8.26	173.81±36.95	120.20±35.32	a) <0.01** b) <0.01** c) <0.01**
Insulin(μlU/ml)	6.89±4.71	17.53±10.94	9.99±3.62	a) <0.01** b) 0.459NS c) <0.01**
HOMA IR	2.39 ±0.82	8.03 ± 4.85	3.03 ±1.21	a) <0.01** b) 0.438NS c) <0.01**
HOMA-β	145.58±36.51	111.42±43.88	130.42±24.85	a) <0.01** b) <0.01** c) <0.01**

Parameters	Control Mean± SD No.30	Patients Pre-cath. Mean±SD	Patients Post-cath. Mean±SD	P- value
TG mg/dL)	106.40±19.73	257.34±87.12	197.22±35.36	a) <0.01** b) <0.01** c) <0.01**
TC (mg/dL)	164.13±16.40	276.40±43.82	246.06±42.79	a) <0.01** b) <0.01** c) <0.01**
LDL.c(mg/dL)	88.95±14.90	183.71±43.89	165.36±44.79	a) <0.01** b) <0.01** c) 0.115NS
VLDL.c(mg/dL)	21.28±3.95	52.51±16.63	40.05±12.98	a) <0.01** b) <0.01** c) <0.01**
HDL.c(mg/dL)	53.90±7.54	38.09±8.46	39.47±7.19	a)<0.01** b) <0.01** c) 0.497NS
Afamin(pg/mL)	1.95±0.26	1.46±0.53	1.94±0.33	a)<0.01** b) 0.816NS c) <0.01**
Preptin(pg/mL)	41.58±23.50	85.73±41.97	58.93±23.99	a) <0.01** b) <0.01** c) <0.01**

a) Significant difference between values in Control and Patients (Pre- catheterization). b) Significant difference between values in Control and Patients (Post- catheterization). c) Significant difference between values in Patients (Pre- catheterization) and Patients (Post- catheterization), BMI: Body mass index, NS =non-significant at the› 0.05 level, Data represented as Mean ±SD, SD: Stander deviation, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, **=significant differences at 1%, NS =non-significant at the 0.05 level, FBG: fasting blood glucose, HOMA-IR: Homoeostasis model assessment-insulin resistance. TG: triglyceride, TC: total cholesterol,HDL-c :high density lipoprotein-cholesterol, LDL :low density lipoprotein, VLDL.c: Very low density lipoprotein- cholesterol.

Hypercholesterolemia and triglyceridemia consider independent risk factors that alone or composedcan hasten the advancement of CVD and development of atherosclerotic lesions. HDL may be defensive by reversible cholesterol abiggerelevation of LDL may also cause a bigger decrease of HDL because there is reciprocal correlation between the concentration of LDL and HDL [16] .

Table (2): Results of univariate analysis of Afamin level with investigated biochemical parameters in the patients group

Variables	r	P
Age (Year)	-0.229	0.233
BMI (kg/m2)	-0.008	0.964
SBP (mm/Hg)	0.040	0.513
DBP (mm/Hg)	0.042	0.6 42
FBG (mg/dL)	-0.221	0.240
Insulin (µIU/mL)	0.122	0.522
HOMA-IR	-0.490	0.037*
HOMA-β	0.073	0.706
TG (mg/dL)	-0.098	0.05*
TC (mg/dL)	-0.306	0.101
LDL-C (mg/dL)	-0.370	0.038*
VLDL-C (mg/dL)	-0.025	0.047*
HDL-C (mg/dL)	0.246	0. 541
Preptin (pg/mL)	-0.321	0.048*

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

Table (3): Results of univariate analysis of Preptin level with investigated biochemical parameters in the patients group

Parameters	r	P
Age (Year)	0.031	0.612
BMI (kg/m2)	0.304	0. 031*
SBP (mm/Hg)	0.052	0.421
DBP (mm/Hg)	0.131	0.150
FBG (mg/dL)	0.065	0.731
Insulin (μIU/mL)	0.20	0.001*
HOMA-IR	0.390	0.001*
HOMA-β	-0.341	0.01*
TG (mg/dL)	0.094	0.620
TC (mg/dL)	0.010	0.960
LDL.c (mg/dL)	-0.018	0.924
VLDL.c (mg/dL)	0.145	0.446
HDL.c (mg/dL)	-0.313	0.092
Afamin	-0.321	0.048*

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

Also, noticed the Afamin levels are a significantly elevated (P<0.01) in patients post- catheterization compared with the pre- catheterization. In the study ofclinical assay evaluation, middle Afamin concentrations were only a bit decreased in patients with heart failure. Patients with pneumonia, heart failure and co-morbidity of pneumonia, in addition tosepsis showed markedly reduced Afamin concentrations. Although the physiological properties of this protein are not fully characterized, many lines of evidence now indicate that Afamin possesses vitamin E-binding properties, which play a crucial role in protection against oxidative damage [17].

In addition, Afamin is known to be expressed mainlyin the liver and to be abundant not only in human serum it is also in the extra vascular fluids like follicular, seminal, and cerebrospinal fluids, suggesting that it plays a role in fertility and neuroprotection [18].

Verylately, the human plasma vitamin E-binding protein Afamin was statedto be very significantly related with criteria for metabolic syndrome in three independent human overall populations. The study by (Kronenberg *et al.*) likewise found that Afamin most intensely connected with triglycerides and waist perimeterin older populations of femalesandmales[19].

Also, illustrated the Preptin levels in patients were significantly decreased (P<0.01), during post-catheterization, compared with the pre- catheterization. The elevation or decreases in the circulatory Preptin amount were found associated with Insulin levels in people [20].

Preptin was found to improve Insulin secretion following glucose stimulation in cultured b-cells, in the secludedperfused rat pancreas[21] . Preptin is thoughtto be a physiological Insulin secretion enhancer induced by glucose. statedthat the circulating level of Preptin was 398 ± 13 ng/L in normal-weight persons, with levels in menbeing lower than in women. Studies have high Preptin levels in patients with metabolic disorders including gestational diabetes mellitus, type 2 diabetes mellitus, polycystic ovary syndrome, and impaired glucose tolerance [6].

Conclusion

In conclusion, The present study concluded for the first time that decreased Afamin and increased preptin levels are strongly involved in the progress of cardiovascular diseases and could independently predict the improvement of the pathogenesis of cardiovascular disease in CVD patients treatment by catheterization . They were associated with atherosclerosis disease that was considered one of the most important leading causes of CVD. The fundamental molecular mechanisms of Afamin and Preptin, on CVD still have not been clarified. This requires deeper investigation.

Acknowledgement: The writers would like to thank all the participated patients and all the staff of the "open heart Unit" at "AL-Sader Teaching Hospital" in Najaf Province-Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding.

Reference

1. Air, Labrosciano C T Tavella, R Beltrame J F & Ranasinghe I . Readmissions following hospitalisations for cardiovascular disease: a scoping review of the Australian literature. Aust. Heal. Rev. (2019).

2. Sugamura K & Keaney JrJ F. Reactive oxygen species in cardiovascular disease. Free Radic. Biol. Med.51, 978–992 (2011).
3. Ayanian J Z & Epstein A M. Differences in the use of procedures between women and men hospitalized for coronary heart disease. N. Engl. J. Med.325, 221–225 (1991).
4. Aad Get al. Muon recontruction performance of the ATLAS detector in proton–proton colliion data at $$\qrt {} $$= 13 TeV. Eur. Phys. J. C76, 292 (2016).
5. Zeiner M Set al. Trocar seal assembly. (2016).
6. Celik Oet al. An appraisal of serum preptin levels in PCOS. Fertil. Steril.95, 314–316 (2011).
7. BUCHANAN C M. PHILLIPSA R J. & COOPER G J S. Preptin derived from proinsulin-like growth factor II (proIGF-II) is secreted from pancreatic islet β-cells and enhances insulin secretion. Biochem. J.360, 431–439 (2001).
8. Li Net al. Lower circulating preptin levels in male patients with osteoporosis are correlated with bone mineral density and bone formation. BMC Musculoskelet. Disord.14, 49 (2013).
9. Canoy D & Buchan I . Challenges in obesity epidemiology. Obes. Rev.8, 1–11 (2007).
10. Aravind P & Prasad M N V. Cadmium-induced toxicity reversal by zinc in Ceratophyllum demersum L.(a free floating aquatic macrophyte) together with exogenous supplements of amino- and organic acids. Chemosphere61, 1720–1733 (2005).
11. Members W Get al. Executive summary: heart disease and stroke statistics—2010 update: a report from the American Heart Association. Circulation 121, 948–954 (2010).
12. Yusoff K. Vitamin E in cardiovascular disease: has the die been cast? Asia Pac. J. Clin. Nutr.11, S443–S447 (2002).
13. Hansson G K. Libby P & Tabas I. Inflammation and plaque vulnerability. J. Intern. Med.278, 483–493 (2015).
14. Kreisberg R A & Reusch J E B. Hyperlipidemia (high blood fat). J. Clin. Endocrinol. Metab.90, b (2005).
15. Baqer H. Gumar A S & Alsafy B M. Diagnosis of Atherosclerosis by using digital image processing. Univ. Thi-Qar J.12, 119–126 (2017).
16. Shameer P. Prasad PVS & Kaviarasan PK . Serum zinc level in vitiligo: a case control study. Indian J. Dermatology, Venereol. Leprol.71, 206 (2005).
17. Voegele A F et al. Characterization of the vitamin E-binding properties of human plasma afamin. Biochemistry41, 14532–14538 (2002).
18. JerkovicL et al. Afamin is a novel human vitamin E-binding glycoprotein characterization and in vitro expression. J. Proteome Res.4, 889–899 (2005).
19. Kronenberg F et al. Plasma concentrations of afamin are associated with the prevalence and development of metabolic syndrome. Circ. Cardiovasc. Genet.7, 822–829 (2014).
20. Caf F Algul S & KoyunM. Investigation of the irisin, preptin and adropin levels in the blood serum of Alburnus tarichi. Cell Mol Biol (Noisy le Gd.63, (2017).
21. ChengKCet al. Characterization of preptin-induced insulin secretion in pancreatic-cells. J. Endocrinol. 215, 43 (2012).