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Serum Concentration of Apelin (AP) and Asymmetric Dimethylarginine (ADMA) in Hypertensive Patients on Different Modalities of Treatment.

Hipertansif Hastalara Uygulanan Farklı Tedavi Metodlarının Apelin (AP) ve Asimetrik Dimetilarjinin (ADMA) Serum Konsantrasyonları Üzerine Etkisinin Değerlendirilmesi

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ABSTRACT

Purpose: Hypertension (HTN) is considered a major health problem. Apelin (AP) a novel multifunction peptide implicated in regulation of the cardiovascular system, including blood pressure and cardiac function control, Evidence has accumulated that asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide (NO) synthase. ADMA inhibits vascular NO production at concentrations found in pathophysiological conditions. However, there is no data about ADMA and apelin levels in essential hypertension and any relationship between them and the effect of antihypertensive drugs from various classes on these parameters of endothelial function. The aim of this study is to assess the status of Apelin and Assymetric DiMethyl Arginine in essential Hypertension on various modalities of treatment.

Methods: The present study is a cross-sectional study (2007/2008) at Al-Yarmouk Teaching Hospital. Includes measurement of serum AP and ADMA in patients with hypertension. a total of 80 patients with HTN were involved in this study, they were classified according to modality of treatment as Hypertensives on captopril G1: (n=40); Hypertensives on atenolol G2: (n=40). A matching group of eighty apparently healthy volunteers who were included as controls (n=80.

Results: Serum AP was significantly reduced and serum ADMA was significantly elevated in hypertensive patients (G1 & G2) as compared with controls (G3) ($p < 0.001$), also the above significant alteration in these two parameters was found when hypertensives on atenolol (G2) were compared with hypertensives on captopril (G1) ($p < 0.001$). AP was negatively correlated with ADMA ($r = -0.9$, $p<0.05$ for G1) ($r = -0.8$, $p<0.05$ for G2); however, this correlation was lost in control group.

Conclusion: Patients with HTN have high level of serum ADMA associated with low level of AP compared with controls, both AP and ADMA were negatively correlated; this was most pronounced in G2 indicating positive effects of captopril on NO pathway.

Key words : hypertension, Apelin, asymmetric dimethyl arginine, captopril, atenelol

ÖZET

Amaç: Hipertansiyon çok önemli bir sağlık sorunudur. Apelin (AP); kardiyovasküler sistemin düzenlenmesinin yanı sıra kan basıncı ve kardiak fonksiyonlarının kontrolünde rol aldığı düşünülen yeni bir multifonksiyonel peptidtir. Mevcut kanıtlar Asimetrik Dimetilarjinin (ADMA)'nin nitrik oksit (NO) sentaz inhibitörü ile rekabet halinde olan endojen bir peptit olduğunu göstermektedir. Patofizyolojik konsantrasyonlarda vasküler nitrik oksit (NO) üretimini baskılayan ADMA inhibitörleri bulunmuştur. Fakat esansiyel hipertansiyonda ki ADMA ve apelin seviyeleri ve bunların birbirleriyle olan ilişkileri ve farklı sınıflardan antihipertansif ilaçların endoteliyal fonksiyonları kapsayan bu parametrelerin üzerine olan etkileri hakkında henüz net bir bilgi yoktur. Bu çalışmada esansiyel hipertansiyon tedavisinde kullanılan çeşitli yöntemlerde ki ADMA ve apelinin düzeylerinin değerlendirilmesi amaçlanmıştır.

Yöntem: Bu araştırma Al-Yarmouk Eğitim Hastanesinde 2007-2008 yıllarını kapsayan dönemsel bir çalışmadır. Çalışmamız hipertansif hastalarda AP ve ADMA düzeylerinin belirlenmesini içermektedir. 80 hipertansif hasta (G1 ve G2 grupları) bu çalışmaya dahil edilmiş olup

uygulanan tedavi metoduna göre sınıflandırılmıştır; kaptopril tedavisi uygulananlar, G1 (n=40); ve atenolol tedavisi uygulananlar, G2 (n=40). Hasta sayısına denk sağlıklı (n=80) bireylerde control grubunu oluşturmuştur.

Bulgular: Hipertansif hastalar (G1 ve G2) kontrol grubu (G3) ile kıyaslandığında, serum AP seviyelerinde önemli derecede azalma, serum ADMA seviyelerinde ise önemli oranda yükselme olduğu belirlenmiştir (p< 0.001). Ayrıca hipertansif tedavi metodları; atenolol (G2) ve captopril (G1) uygulanan iki hasta grubu kıyaslandığında bu iki parametre arasında da önemli farklılıklar bulunmuştur (p< 0.001). AP'nin, ADMA ile negatif bir korelasyon gösterdiği bulunmuştur. (G1 için; r = -0.9, p<0.05) (G2 için; r = -0.8, p<0.05). Fakat bu korelasyon kontrol grubunda gözlenmemiştir.

Sonuç:Hipertansiyonlu hastalarda, kontrol grubuna göre serum ADMA seviyesi yüksek, serum AP seviyesi ise düşüktür. Aynı zamanda AP ve ADMA arasında negatif bir ilişki vardır. G2'de belirgin olan bu durum, kaptoprilin NO yolağı üzerinde pozitif etkileri olduğunu göstermektedir.

Anahtar Kelimeler: Hipertansiyon, apelin, asimetrik dimetilarjinin, kaptopril, atenelol

Introduction

Hypertension (HTN) is considered a major health problem that emerges from the wide occurrence of its cardiovascular complications[1].

Apelin (AP) a novel multifunction peptide implicated in regulation of the cardiovascular system, including blood pressure and cardiac function control - has been postulated to be involved in the pathophysiology of hypertension and hypertensive heart disease[2].

Evidence has accumulated that asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide (NO) synthase. ADMA inhibits vascular NO production at concentrations found in pathophysiological conditions; it also causes local vasoconstriction when infused intra-arterially. ADMA is increased in the plasma of humans with hypercholesterolemia, atherosclerosis, hypertension, chronic renal failure, chronic heart failure, and other clinical conditions. Increased ADMA levels are associated with reduced NO synthesis as assessed by impaired endothelium-dependent vasodilation or reduced NO metabolite levels[3].

Both apelin and asymetric dymethyl arginine (ADMA) regulate blood pressures. Low apelin and high ADMA levels have been reported in several cardiometabolic disorders[4]. However, there is no data about ADMA and apelin levels in essential hypertension and any relationship between them and the effect of antihypertensive drugs from various classes on these parameters of endothelial function. This study was conducted to assess the status of AP and ADMA in eHTN on various modalities of treatment.

Subjects & Methods

A-Subjects

The study was a cross-sectional study carried out during the period from October, 2007 till the end of September, 2008. The protocol for the study was approved by the Ethical committee of Medical College, and informed signed consent was given by each subject.

B. Blood samples:

Five milliliters of random venous blood were withdrawn from each patient, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendrof tube and was used for measurement of AP and ADMA. The tubes were stored at –20o C until analysis, which was done within one month after collection[5,6].

C-Methods

Measurement of serum AP and ADMA was done by ELISA (Enzyme-linked immunosorbent assay) Kits manufactured by USA

Phoenix Pharmaceuticals, Inc.

D. Statistical analysis:

Statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significancy of mean difference, a correlation was made between the studied parameters. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results

A-Subjects

A total of 80 patients with essential HTN (eHTN) were enrolled in this study; forty of them (G1) were on ACE inhibitor (captopril 25-150 mg/day) age range 32-74 years (mean age = 52.3 + 6.8 year), equal sex distribution; systolic arterial blood pressure (sBP) ranges between 125-185 mmHg, mean sBP + SD was 148.4 +

12.1 mmHg; diastolic arterial blood pressure (dBP) ranges between 77 -12.5 mmHg, mean dBP + SD was 89.4 + 6.2 mmHg ; triglyceride (TG) ranges between 1.5-2.5 mmol/L, mean TG + SD was 1.6 + 0.8 mmol/L; low-density lipoprotein (LDL) ranges between 1.5-3.5 mmol/L, mean LDL + SD was 3.2 + 1.4 mmol/L; total cholesterol (TC) ranges between 2.5-7.5 mmol/L, mean TC + SD was 5.2 + 2.8 mmol/L; high-density lipoprotein (HDL) ranges between 1.1-2.1mmol/L, mean HDL + SD was 1.5 + 0. 3 mmol/L; very low-density lipoprotein (VLDL) ranges between 0.3-0.5 mmol/L, mean VLDL + SD was 0.4 + 0. 1 mmol/L atherogenic index (LDL/HDL)(IA) ranges between 2.5-3.5, mean AI + SD was 3.1 + 0.6; LDL size index (TG/HDL)(LDL-SI) ranges between 0.5-2.6 mmol/L, mean LDL-SI + SD was 1.7+ 0.9 as in Table 1.

Other forty of hypertensive patients (G2) were on β-blocker (atenolol 50-100 mg/day), age range 30-70 year (mean age = 50 + 7.5 year), equal sex distribution; systolic arterial blood pressure (sBP) ranges between 135-165 mmHg, mean sBP + SD was 152.2 ± 10.5 mmHg; diastolic arterial blood pressure (dBP) ranges between 85-100 mmHg, mean dBP + SD was 90.5 ± 7.2 mmHg; triglyceride (TG) ranges between 1.8-4.8 mmol/L, mean TG + SD was 3.8 ± 1 mmol/L; low-density lipoprotein (LDL) ranges between 1.9-4.7 mmol/L, mean

LDL + SD was 3.2 + 1.2 mmol/L; total cholesterol (TC) ranges between 3.2-7.4 mmol/L, mean TC + SD was 4.8 + 0.8 mmol/L; high-density lipoprotein (HDL) ranges between 0.9-1.1mmol/L, mean HDL + SD was 0.9 + 0.2 mmol/L; very low-density lipoprotein (VLDL) ranges between 0.4-1mmol/L, mean VLDL + SD was 0.7 + 0. 2 mmol/L; atherogenic index (LDL/HDL)(IA) ranges between 2.3-4.7, mean AI + SD was 3.6 + 1.1; LDL size index (TG/HDL)(LDL-SI) ranges between 1.5-6.6 mmol/L, mean LDL-SI + SD was 3.7+ 1.9 as in Table 1.

Control group (G3):

Eighty apparently healthy subjects were involved as a control group (G3) with matching age and sex to the patient group.

Age range 28-65 year (mean age = 48.2 + 6.4 year), equal sex distribution; systolic arterial blood pressure (sBP) ranges between 110-135 mmHg, mean sBP + SD was 112.2 ± 6.5 mmHg; diastolic arterial blood pressure (dBP) ranges between 60-80 mmHg, mean dBP + SD was 70.5 ± 5.2 mmHg; triglyceride (TG) ranges between 0.4-2.1 mmol/L, mean TG + SD was 1.8 ± 0.4 mmol/L; low-density lipoprotein (LDL) ranges between 3.3-4.5 mmol/L, mean

LDL + SD was 2.2 + 0.7 mmol/L; total cholesterol (TC) ranges between 7.2-5.4 mmol/L, mean TC + SD was 4.4 + 0.8 mmol/L; high-density lipoprotein (HDL) ranges between 0.6-1.7mmol/L, mean HDL + SD was 1.3 + 0.4mmol/L; very low-density lipoprotein (VLDL) ranges between 0.08-0.4mmol/L, mean VLDL + SD was 0.35 + 0. 08 mmol/L; atherogenic index (LDL/HDL)(IA) ranges between 0.3-4, mean AI + SD was 2.6 + 1.1; LDL size index (TG/HDL)(LDL-SI) ranges between 1.2-6.6 mmol/L, mean LDL-SI + SD was 1.2+ 0.5 as in Table 1.

Any subject with other medical illnesses that may have an effect on the measured parameters was excluded from the study, as cardiac, hepatic, endocrine, metabolic diseases, smoking and alcoholism. None of the female patients were pregnant nor on contraceptive pills.

B-Serum AP & ADMA:

Serum AP was significantly reduced accompanied by significant increase in ADMA in HTN patients (G1, G2) compared with healthy controls (G3) [P < 0.001 for both] with the most significant alteration in patients on atenolol therapy (G2) even when compared with hypertensive patients on captopril (G1) [P < 0.001 for both] as in *Table 2*. Neither parameters (AP & ADMA) were not correlated with BP nor with any elements of lipid profile, but AP negatively correlated with ADMA (r = -0.9, p<0.05 for G1) (r = -0.8, p<0.05 for G2) as in *Figure 1, 2 respectively*; however, this correlation was lost in control group.

Discussion

Under normal circumstances, vascular tone is influenced by adipokines. However, it is thought that vascular tone regulation is compromised in obesity and obesity-related disorders, in which the amount of adipose tissue has grown out of proportion[7].

This eventually leads to a dysregulated synthesis of vasoactive adipokines by dysfunctional adipose tissue in favour of harmful proinflammatory adipokines (for example, leptin)[8].

The dysregulated macrophages into adipose tissue, possibly as a result of monocyte chemoattractant protein (MCP)-1[9] and leptin[10] release from adipocytes, lead to a state of inflammation within adipose tissue. A proinflammatory state in adipose tissue can induce not only a dysregulation of vascular tone but also local insulin resistance,adhesion of monocytes, vascular remodelling, foam cell formation in the arterial wall and endothelial dysfunction[9].

Table.1. Clinical criteria of of patients` groups with essential Hypertension & Control (presented as range and mean ± SD).

Group	G1	G2	G3
No	40	40	80
Age / year (Mean ± SD)	52.3 ± 6.8	52 ± 8	54 ± 6
Age range (years)	32-74	30-70	28-65
Sex	Equal distribution		
sBP rage (mmHg)	125-185	135-165	110-135
sBP (Mean ± SD) (mmHg)	148.4 ± 12.1	152.2 ± 10.5	112.2 ± 6.5
dBP rage (mmHg)	77-12.5	85-100	60-80
dBP (Mean ± SD) (mmHg)	89.4 ± 6.2	90.5 ± 7.2	70.5 ± 5.2
TG (mmol/L) (mean ± SD)	1.6 ± 0.8	3.8 ± 1	1.8 ± 0.4
TG Range(mmol/L)	1.5-2.5	1.8-4.8	0.4-2.1
LDL-C (mmol/L) (mean ± SD)	3.2 ± 1.4	3.2 ± 1.2	2.2 ± 0.7
LDL-C Range(mmol/L)	1.5-3.5	1.9-4.7	3.3-4.5
TC (mmol/L) (mean ± SD)	5.2 ± 2.8	3.2-7.4	4.4 ± 0.8
TC Range(mmol/L)	2.5-7.5	4.8 ± 0.8	5.4-7.2
HDL (mmol/L) (mean ± SD)	1.5 ± 0. 3	0.9 ± 0.2	1.3 ± 0.4
HDL Range(mmol/L)	1.1-2.1	0.9-1.1	0.6-1.7
VLDL-C (mmol/L) (mean ± SD)	0.4 ± 0. 1	0.7 ± 0. 2	0.35 ± 0. 08
VLDL-C Range(mmol/L)	0.3-0.5	0.4-1	0.08-04
Atherogenic Index (mean ± SD)	3.1 ± 0.6	3.6 ± 1.1	2.6 ± 1.1
Atherogenic Index Range(mmol/L)	2.5-3.5	2.3-4.7	0.3-4
LDL Size Index (mean ± SD)	1.7± 0.9	3.7± 1.9	1.2± 0.5
LDL Size Index Range	0.5-2.6	1.5-6.6	1.2-6.6

(G1): Hypertensive patients on captopril treatment.

(G2): Hypertensive patients on atenolol treatment.

(G3): Healthy Controls.

Table.2. The mean serum apelin (AP) & asymmetric dimethyl arginine (ADMA) in different hypertensive and control groups (presented as mean + SD).

Variable	G1	G2	G3
s.ADMA (µmol/L)	1.8 + 0.5*	2.1 + 0.4*§	0.4 + 0.2
s.AP (µmol/L)	0.5 + 0.1*	0.7 + 0.2*§	2.4 + 0.6

(G1): Hypertensive patients on captopril treatment.

(G2): Hypertensive patients on atenolol treatment.

(G3): Healthy Controls.

* t-test: G1, G2 versus G3 $p < 0.001$

§ t-test: G2 versus G1, $p<0.05$

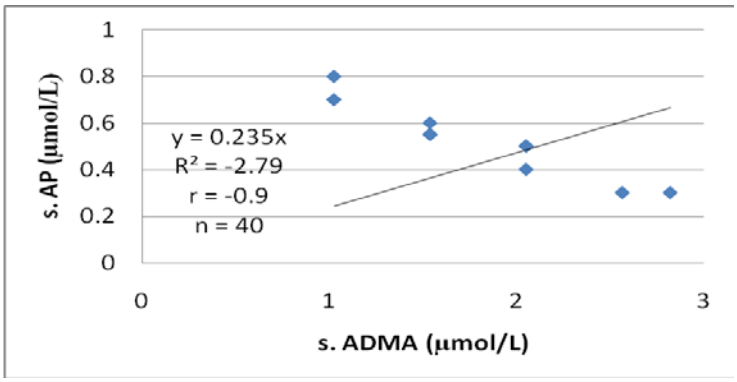

Figure 1. Correlation between s. AP & s. ADMA in G1: hypertensives on captopril (n=40; r = - 0.9; P< 0.05).

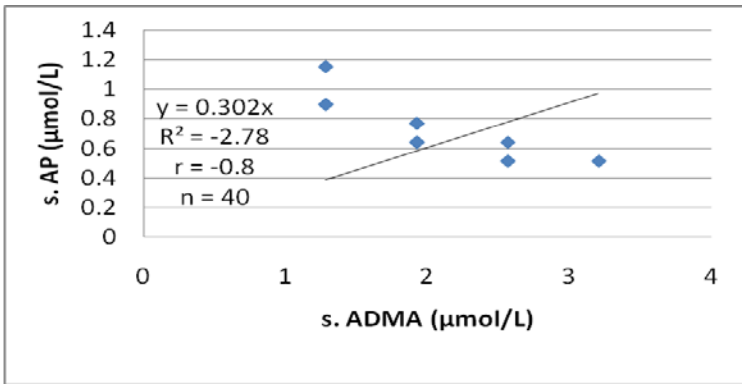

Figure 2. Correlation between s. AP & s. ADMA in G2: hypertensives on atenolol (n=40; r = - 0.8; P< 0.05).

Endothelial dysfunction is reflected as a decrease in nitric oxide (NO) bioavailability, endothelium-dependent relaxation and impaired ability of the endothelium to respond to circulating hormones. All of these changes clearly promote the development of cardiovascular diseases[10].

Apelin, of which different isoforms exist, acts through the binding to a specific G protein-coupled receptor named APJ[11], which is present on endothelial cells, vascular smooth muscle cells and cardiomyocytes[12].

In the presence of functional endothelium, this vasoconstrictive effect may be counterbalanced or even masked by activation of APJ receptors on vascular endothelial cells, resulting in the release of endothelial vasodilator substances such as NO[12]. All of these data taken together suggest a role for the apelin-APJ system as a regulator of vascular tone.Apelin causes NO-dependent vasorelaxation of human arteries both in vitro and in vivo[12,13]. In vivo exogenous apelin administration has been shown to cause a rapid NO-dependent fall in blood pressure in a rodent model, confirming its powerful vasorelaxing effect[13].

Apelin production in adipose tissue is strongly upregulated by insulin, and plasma concentrations are increased in obese and hyperinsulinemic mice and humans[14].

Only few animal studies on the effect of ADMA in vivo exist. These studies describe the dose-dependent pressor and bradycardic effects of ADMA infusion as a short-term effects [15], [16]; however, long-term vascular effects of ADMA infusion were also exist (like significant coronary microvascular lesions)[17]. These long-term effects were not mediated solely by inhibition of endothelial NO synthesis, but presumably also by direct upregulation of angiotensin-converting enzyme (ACE), and increased oxidative stress through angiotensin 1 receptor also appears to be involved in this process[18].

The results showed that, blood pressure is regulated by a balance between AP (through its positive effect on nitric oxide (NO)) and ADMA (an endogenous inhibitor of NO), NO consider the major determent of vascular tone[19] this was supported by significant

alteration in serum level of these metabolites in hypertensive patients on different treatment as compared with controls which can be compared with previous studies like[19,20]. The significant alteration between hypertensive groups was attributed to fact that ACE inhibitor reduces oxidative stress and improves the NO pathway in patients with essential hypertension with a possible vasculoprotective effect of the compound in retarding vascular dysfunction and atherogenesis that often develops rapidly in hypertensive patients[21,22]. Data on effects of other antihypertensive drugs on NO pathway are scanty which necessate future research.

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