**Investigation of plasma apelin level in patients with essential hypertension corresponding to the type of obesity.**

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Obesity has been consistently associated with hypertension and increased cardiovascular risk. Based on population studies risk estimates indicate that at least two-thirds of the prevalence of hypertension can be directly attributed to obesity. Although BMI as a measure of obesity is a good predictor of all-cause and cardiovascular mortality, as recently described in two separate meta-analyses [1, 2], overall mortality and especially cardiovascular mortality seems to be better predicted by abdominal or central obesity in addition to BMI [3-5].

Obesity-related hypertension is commonly associated with further elements of the metabolic syndrome, such as hyperinsulinemia and glucose intolerance. In particular, one should be aware that diabetes de novo occurs in 2 % of treated hypertensive patients per year [6].

It’s well known that weight gain is associated with sustained inflammatory response with accompanied adipokine dysregulation which leads to chronic inflammation as well as insulin resistance. Chronic low-grade inflammation is thought to be the key parameter in the development of IR and T2DM. Apelin, a recently described adipokine, has been shown to have pro-inflammatory role with a close correlation demonstrated between apelin and TNFα levels, as well as other pro-inflammatory adipokines [7, 8].

This highlights the importance of the site of deposition of adipose tissue in estimation of cardiovasculat risk in obese hypertensive patients.

Aim of the study: to investigate apelin’s activity in patients with essential hypertension with obesity according to the type of obesity.

Materials and methods: 96 patients with EH were examined. Inquiring, inspection and laboratory investigations were provided according to the recommendations of Ukrainian Society of Cardiology and ESC/ESH recommendations 2007/2009 [9].The study was approved by local institutional review board committees, and all participants provided written informed consent. All subjects underwent measurements of height, weight at the baseline visit. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m2). Waist circumference (WC) was measured at the level of the umbilicus, using an unstretched tape meter, without any pressure to body surface over light clothing. Visceral obesity was estimated according to the ESC/ESH recommendations 2009 [9].

Three measurements of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken using a standardized sphygmomanometer on the right arm, after a 15-minute rest in a sitting position; the average of the three measurements was used as subject’s blood pressure.

A blood specimen was collected after overnight fasting into a tube with further centrifuging and freezing for investigations. Carbohydrate metabolism was evaluated on the basis of plasma glucose, insulin, glycated haemoglobin (HbA1c) that were measured as at fasting, as after 120 min of standard glucose tolerance test (OGTT). For insulin measurements the laboratory set DRG® Insulin (DRG Instruments GmbH, Germany, Marburg) was used. Glucose and lipid profile (total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol levels (HDL-C)) were determined using standard biochemical methods.

Low density lipoprotein-cholesterol was calculated (LDL-C) with W.T. Friedewald formula [10]: LDL-C = TC —( HDL-C + TG / 2,22), where TG / 2,22 is very low density lipoprotein-cholesterol.

Index of atherogenity (IA) was calculated according A. M. Klimov fomula [11]:

IA = (TC — HDL-C) / HDL-C 2

Apelin was estimated in blood plasma using ELISA technique (Phoenix, USA).

Statistical representation of the results is median (Me) and inter-quartile range. All patients were categorized according to cluster analysis using k-means using apelin and BMI means. Difference between groups was calculated using Kruskal-Wallis test. A p value of less than 0.05 was considered to be statistically significant.

**Results**

Comparing with control group the average means of BMI and apelin level in total group (96 pts) were significantly higher: 30,47 (27,70; 33,70) kg/m2 and 0,28 (0,16; 0,48) ng/ml respectively. In comparing control group: BMI – 21,23 (18,96; 23,12) kg/m2and apelin – 0,12 (0,10; 0,15) ng/ml. 77,1 % of hypertensive patients had visceral obesity.

To find out the interrelations of obesity, adipose tissue location and expression of adipokine apelin, all patients were categorized into 4 cluster groups based on k-means according apelin and BMI data (see pic. 1).



Picture 1 – Clustering of results according apelin and BMI data.

There were 23 pts. with EH In the 1st cluster of 40-71 age, Me - 63,0y.o.; 13 females and 10 males. 34,7 % of the patients had visceral obesity. The 2nd cluster consists of 22 pts. With EH of 35-72 age, Me – 60,5 y.o.; 12 females and 10 males. And 95,4 % from the group had abdominal type of obesity. 3rd cluster includes 14 pts. with EH of 54-74 age, Me – 61,5 y.o.; 8 females and 6 males. 85,7 % of the patients had abdominal distribution of adipose tissue. In the 4th cluster there were 37 pts of 30-72 age, Me – 58,0y.o. 89,1 % of the patients had visceral type of obesity.

The baseline characteristics of EH duration, blood pressure data, anthropometric measurements, results of carbohydrate and lipid pool investigation are shown in Table 1.

**Table 1**

**Results of inquiring, anthropometric measurements, laboratory and instrumental investigations data in patients with essential hypertension according to clusters distribution**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  GroupsMeans | 1 Cluster,23 pts with EH | 2 Cluster,22 pts with EH | 3 Cluster,14 pts with EH | 4 Cluster,37 pts with EH | Kruskal-Wallis ANOVA; Median Test |
| Duration of EH, years | 8,0 (5,0;12,0) | 10,0 (6,0;13,0) | 11,5 (5,0; 13,0 | 12,0 (6,0; 17,0) | p>0,05 |
| SBP, mm Hg | 160 (150;180) | 180 (160;185) | 166 (160;180) | 160 (150;165) | p<0,05 |
| DBP, mm Hg | 90 (90;100) | 100 (90;100) | 99 (89;100) | 95 (90;100) | p>0,05 |
| BMI, kg/m2 | 26,09 (25,15;27,15) | 35,82 (34,92;37,12) | 29,50 (26,00;30,40) | 31,21 (29,70;32,89) | p<0,05 |
| WC, cm | 88,00 (84,10;96,00) | 111,20 (106,10;120,20) | 100,50 (95,00;106,40) | 102,00 (94,00;112,50) | p<0,05 |
| HC, cm | 99,00 (97,00;104,10) | 122,00 (113,00;125,10) | 108,50 (103,00;112,40) | 110,00 (105,00;112,00) | p<0,05 |
| Apelin, ng/ml | 0,29 (0,16; 0,38) | 0,37 (0,23; 0,64) | 0,87 (0,68; 1,00) | 0,18 (0,14; 0,25) | p<0,01 |
| IL-6, pg/ml | 13,35 (8,77; 19,63) | 9,81 (8,79; 11,82) | 8,95 (7,62; 26,00) | 13,47 (10,00; 15,64) | p>0,05 |
| TC, mmol/l | 5,21 (4,63; 5,60) | 4,95 (4,02; 4,90) | 5,47 (4,29; 6,00) | 5,49 (4,98; 6,30) | p<0,05 |
| TG, mmol/l | 1,52 (1,11; 2,67) | 1,45 (0,83; 2,39) | 1,12 (0,80; 1,98) | 1,62 (1,11; 2,73) | p>0,05 |
| HDL-C,mmol/l | 1,23 (0,88; 1,28) | 1,20 (0,74; 1,35) | 1,12 (0,69; 1,33) | 0,76 (0,73; 1,05) | p<0,05 |
| LDL-C,mmol/l | 3,29 (2,29; 3,61) | 2,89 (1,91; 3,57) | 3,41 (2,51; 4,91) | 3,70 (3,44; 4,74) | p<0,05 |
| VLDL-C, mmol/l | 0,66 (0.50; 1,21) | 0,58 (0,38; 1,09) | 0,50 (0,36; 0,89) | 0,77 (0,50; 1,24) | p>0,05 |
| IA | 3,24 (2,70; 5,64) | 3,32 (2,27; 5,54) | 2,80 (2,28; 7,24) | 5,31 (4,15; 7,02) | p<0,01 |
| FPG, mmol/l | 5,51 (4,73; 6,65) | 5,21 (4,90; 7,20) | 6,51 (5,62; 9,55) | 6,90 (5,99; 8,25) | p<0,05 |
| 2h OGGT glucose, mmo/l | 5,96 (5,66; 6,59) | 6,48 (6,32; 7,09) | 5,57 (5,42; 5,72) | 7,13 (6,48; 8,04) | p<0,05 |
| FI, mmol/l | 20,58 (12,47; 26,18) | 19,78 (11,74; 23,22) | 26,5 (18,96; 34,03) | 24,62 (14,10; 29,87) | p>0,05 |
| 2h OGGT insuline, mmo/l | 55,65 (43,68; 59,38) | 67,69 (57,14; 69,18) | 42,87 (40,22; 45,53) | 68,81 (54,48; 80,29) | p<0,01 |
| HOMA | 5,09 (2,19; 6,90) | 4,65 (2,66; 6,65) | 7,38 (4,44; 13,65) | 7,02 (4,51; 9,53) | p<0,05 |
| HbA1c | 7,00 (4,90; 8,00) | 7,15 (6,90; 7,90) | 5,70 (4,77; 9,20) | 7,35 (5,30; 8,10) | p>0,05 |

Data is described by median and inter-quartile range.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; HC, hips circumference; IL-6, interleukine-6;TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; VLDL-C, very low density lipoprotein-cholesterol; IA, index of atherogenity; FPG, fasting plasma glucose. OGGT, oral glucose tolerance test;

Patients in 1st and 2nd clusters had opposite meanings of WC, HC and BMI. But, patients of the 1st cluster had the lowest WC and BMI and also the shortest duration of the disease. It was accompanied with not very pronounced changes in lipid profile, carbohydrate pool and moderate expression of IL-6 and apelin. Comparing with patients of 1st cluster hypertensive obese patients of 2nd cluster had longer anamnesis of EH, dyslipidemia, more pronounced dysglicemia, hypercytokinemia that was accompanied by highest WC, BMI, SAP and DAP and increased level of apelin.

In patients of 3rd and 4th clusters there was no significant difference in BMI data, but there was opposite apelin activity. Level of adipokine in patients of 3rd cluster was 3-fold higher than in other groups. In cluster 4, adipokine’s activity was the lowest one from total amount of patients and in cluster 3 – the highest one. Both groups had similar and longest duration of EH in the whole group. Analysis of the instrumental and laboratory investigations have shown higher levels of SAP and DAP in patients of 3rd cluster in comparing with 4th. On the background of the lowest in the group level of apelin, patients of 4th cluster had significant and highest levels of TC, TG, LDL-C, VLDL-C; lowest data of HDL-C and increasing of IA, almost 2-fold in comparing with patients of other clusters. The most pronounced carbohydrate disorders were common for the patients of 4th cluster. Levels of fasting glucose, post OGTT glucose and insulin, HbA1c, index HOMA were the highest in patients of 4th cluster comparing with other patients with EH. Pronounced hypercytokinemia was established in patients of 4th cluster.

Distribution of the patients according to dysglicemia in each cluster (see pic.2) we found out that, the smallest percentage of accompanied carbohydrate disorders 60,8 % was in hypertensive patients of 1st cluster. In the 2nd cluster there was 68,4 % patients with EH and dysglicemia. Patients of 3d and 4th clusters had hypertension and comorbid carbohydrate pool abnormalities in 85,6 % and 91,8 % correspondingly.

Picture 2 – Essential hypertension and percentage of accompanied dysglycemia in clusters.

There is an evidence that exogenous apelin reduces the peak plasma glucose concentration after a glucose load by increasing glucose turnover through insulin-dependent and -independent pathways. Apelin-deficient animal models have reduced insulin sensitivity and this can be corrected by the administration of exogenous apelin. Dray C, Knauf C, Daviaud D, et al. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metab 2008;8:437–45.

Conversely, exogenous apelin reduces the peak plasma glucose concentration following a glucose load by increasing glucose turnover and this effect is preserved in insulin-resistant animal strains. The exact cellular mechanisms leading to increased glucose uptake are incompletely understood. Apelin increases glucose uptake through phosphorylation of components of insulin-dependent pathways, such as Akt, although increased glucose uptake is still observed in the presence of inhibition of this pathway suggesting both insulin-dependent and -independent pathways.

Yue P, Jin H, Aillaud-Manzanera M, et al. Apelin is necessary for the maintenance of insulin sensitivity. Am J Physiol Endocrinol Metab 2009;298:E59–67.

Other way, clusterization of the hypertensive patients according to the BMI and apelin activity showed also peculiarities of carbohydrate metabolism that is connected with adipokine expression.

Analysis of apelin’s interrelations in total group showed significant correlations with parameters of carbohydrate pool. Numerous positive correlations of apelin were found: with fasting insulin (R=0,29, p<0,05), -post OGTT glucose and insulin levels (R=0,39 and R=0,41 respectively, p<0,05), -HOMA index (R=0,24, p<0,05) and HbA1c (R=0,24, p<0,05). In patients of cluster 1 the significant correlation of apelin and HbA1c was estimated (R=0,53, p<0,05). In patients of 2nd and 4th clusters significant negative correlations of apelin with BMI were detected (R=-0,72 and R=-0,41 respectively, p<0,05).

Interestingly, plasma apelin concentrations are reduced in patients with newly diagnosed type 2 diabetes mellitus but increased in obese non-diabetic individuals. This may suggest that the initial increase in apelin seen in obesity serves to delay the development of type 2 diabetes mellitus by preserving glycaemic control. Li L, Yang G, Li Q, et al. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. Exp Clin Endocrinol Diabetes 2006;114:544–8.

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**Background:** Adiponectin, an anti-inflammatoryadipocytokine, isreducedinhypertension, diabetes, andcoronaryarterydisease (CAD). Arterialstiffness, asaorticpulsewavevelocity (PWV) inhypertensionanddiabetes, andasaugmentationindex (AIx) in CAD, isindependentlyassociatedwithcardiovascularmortality. Weexploredtherelationshipbetweenadiponectinandarterialstiffnessinessentialhypertension.

**Methods:** Seventy-six untreatedpatients, 34 women, aged 47 ± 1 years, mean ± SEM withessentialhypertension, hadbloodpressure (BP), carotid–femoral PWV, AIxplasmaadiponectin, andproinflammatorycytokine C-reactiveprotein (CRP) measuredusing ELISA techniqueafteranovernightfast. Resultswereanalyzedusingunivariateandmultiplelogisticregressionanalysis.

**Results:** There was a significantpositiverelationshipbetweenlogadiponectinandAIx (r = 0.33, P < .005) andplasma HDL-cholesterol (r = 0.40, P < .001). Incontrastthereweresignificantnegativerelationshipswith PWV (r = −0.24, P < .05), transittime (r = −0.37, P < .001), andpulsepressureamplification (r = −0.30, P < .005) inadditiontowaistcircumference, waist-to-hip ratio, height, andweight. In a stepwiseregressionmodel, theindependentpredictorsofAIxwereheartrate, height, meanarterialpressure, age, andgender (R2 = 0.69, P < .0001) withnocontributionfromadiponectin. However, for PWV (R2 = 0.59, P < .0001) theindependentdeterminantsweremeanarterialpressure, age, andadiponectin.

Subjects were categorized into 4 phenotype groups based on the following cut-off points: (1) NWNT (normal waist normal triglyceride, waist circumference <90 cm for men and <80 cm for women; serum triglyceride concentration<1.7 mmol/L); (2) HTG (hypertriglyceridemia, waist circumference <90 cm for men and <80 cm for women;serum triglyceride concentration ≥1.7 mmol/L); (3) EW(enlarged waist, waist circumference ≥90 cm for men and≥80 cm for women; serum triglyceride concentration <1.7