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# INVESTIGATION OF APELIN ACTIVITY IN PATIENTS WITH ESSENTIAL HYPERTENSION AND OBESITY

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Co-morbid hypertension and obesity is a multifactorial disorder, but mechanisms leading to weight gain in hypertensive persons are not completely known. Aim of the study was to investigate apelin's expression in patients with essential hypertension (EH) with obesity in Ukraine patients. Patients were categorized into 4 cluster groups based on k-means according apelin and BMI data. The increased level of peptide apelin in hypertensive patients was detected. Significant dyslipidemia with high atherogene index, dysglicemia, hyperinsulinemia, and pronounced expression of pro-inflammatory cytokine are accompanied with decreasing of apelin level and negative correlation of BMI with peptide.
**Keywords:** essential hypertension, obesity, adipokine, apelin, dislipidemia.

Essential hypertension (EH) stays the important public challenge, because of leading positions in morbidity and mortality not only in Ukraine, but worldwide. Excess body weight is the sixth most important risk factor contributing to the overall burden of disease in the world. Obesity itself is recognized as one of the most important risk factors for the development of hypertension and its progression(Piya MK, 2013).Hypertension in obese patients in over 60% is associated with glucometabolic disturbances, like insulin resistance, glucose intolerance. Moreover, diabetes develops in 2% of treated hypertensive patientsevery year(Demydenko G, 2013).

The Framingham study have shown that future weight gain is significantly greater in hypertensive patients than in normotensive subjects, suggesting that even normal weight hypertensives are at a high risk of developing obesity (Julius S, 2000).

It is clear that co-morbid hypertension and obesity is a multifactorial disorder. But mechanisms leading to weight gain in hypertensive persons are not completely known. It is likely that obesity, hypertension and metabolic abnormalities interact and potentiate their individual impact on cardiovascular risk.

As it's still not possible to identify one mechanism as the dominant aetiological factor, adipokines may have a decisive influence.

Apelin is a recently discovered vasoactive peptide and adipokine that is an endogenous ligand of the APJ receptor and was named 'apelin' after APJ endogenous ligand. This G protein-coupled receptor was identified in 1993, and has a close identity with the angiotensin II type 1 receptor, but does not bind angiotensin-II (Piya MK, 2013).

Apelin and APJ have been found to be expressed in fat tissues, heart and lungs, as well as various regions of the central nervous system. The pathophysiologic action of apelin in obesity remains unclear.

**Aim of the study** was to investigate apelin's expression in Ukraine patients with essential hypertension with obesity.

**Materials and methods:** 96 patients with EHwere recruited in the investigation. Inquiring, inspection and laboratory investigations were provided according to the recommendations of Ukrainian Society of Cardiology and ESC/ESH recommendations 2007/2009 (Mancia G, 2009).The study was approved by local institutional review board committees, and all participants provided written informed consent. All subjects underwent measurements of height, weightat the baseline visit.Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m$^2$).

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 (Little Doctor, Switzerland).

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 Olveks diagnosticum Kit, Russia.

Low density lipoprotein-cholesterol was calculated (LDL-C) with W.T. Friedewald formula (Fukuyama N, 2008):

$$LDL\text{-}C = TC - (HDL\text{-}C + TG / 2{,}22), \quad (1)$$

where TG / 2,22 is very low density lipoprotein-cholesterol.

Index of atherogenity (IA) was calculated according A. M. Klimov fomula(Klimov A N, 1999):

$$IA = (TC - HDL\text{-}C) / HDL\text{-}C \quad (2)$$

Apelin-12 was estimated in blood plasma using ELISA technique (Kit Apelin-12, 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 lessthan 0.05 was considered to be statistically significant.

**Results.** The average means of BMI and apelin level in total group (96 pts) were 30,47 (27,70; 33,70) kg/m$^2$ and 0,28 (0,16; 0,48) ng/ml respectively. 93% of hypertensive patients were overweight. It was significantly higher in comparing with control group: BMI – 21,23 (18,96; 23,12) kg/m2and apelin – 0,12 (0,10; 0,15) ng/ml. To find out the interrelations of excessed fat and expression of adipokine apelin, all patients were categorized into 4 cluster groups based on k-means according apelin and BMI data (see fig. 1).

In the 1st cluster there were 23 pts. with EH of 40-71 age, Me – 63,0 y.o.; 13 females and 10 males. The 2nd cluster consists of 22 pts. With EH of 35-72 age, Me – 60,5 y.o.; 12 females and 10 males. 3rd cluster includes 14 pts. with EH of 54-74 age, Me – 61,5 y.o.; 8 females and 6 males. In the 4th cluster there were 37 pts of 30-72 age, Me – 58,0 y.o.

According to the clusterization the most amount of lean patients were in 1$^{st}$ cluster (21,7%), see

fig. 2. 78% of the patients of 1st cluster were pre obese. The prevalent amount of the 2nd cluster – 59,1%, were hypertensive patients with 2st of obesity. 50% patients of 3rd cluster had obesity of 1st and 45% – were pre obese. In 4th cluster the 70,3% of patients with hypertension had 1st of obesity, and 24, 3% – pre obese.

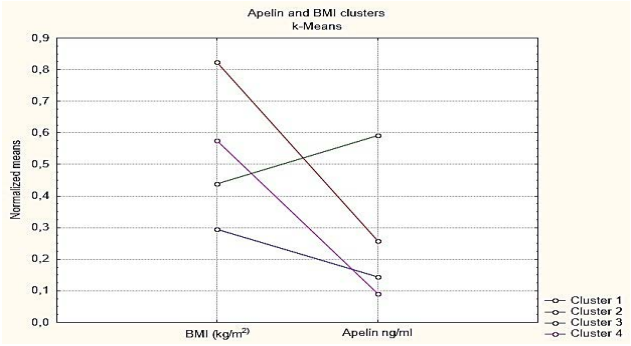


**Fig. 1. Clustering of results according apelin and BMI data**

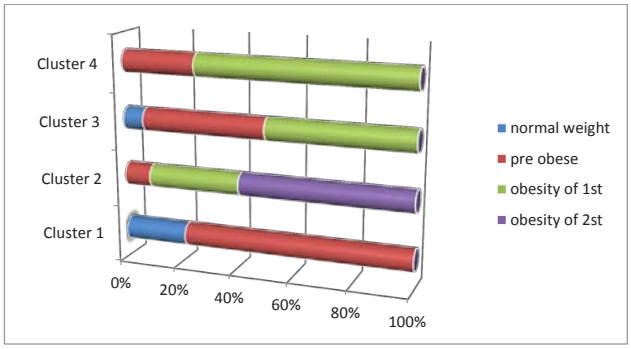


**Fig. 2. Distribution of the patients in the clusters according to the stage of obesity**

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

As it shown, the 1st and 2nd clusters had opposite meanings of BMI. Patients of the 1st cluster had the lowest BMI and also the shortest duration of the disease.

Also it was accompanied with not very pronounced changes in lipid profile, carbohydrate pool and moderate expression of cytokines and adipokine. For the patients of the 2nd cluster the highest BMI, SAP and DAP was common. 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 increased level of apelin.

Despite of not significant difference in BMI data between patients of 3rd and 4th clusters, the opposite apelin activity was detected. In cluster 4, adipokine's activity was the lowest one from total amount of patients and in cluster 3 – the highest one. Level of adipokine in patients of 3rd cluster was 3-fold higher than in other groups. 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 with the prevalence of the pro-inflammatory cytokine – IL-6 was established in patients of 4th cluster. And, obviously the level of plasma apelin depends from not only glucose and insulin expression, but also from components of atherogenesis, such as dyslipidemia with pronounces atherogenic index and pro-inflammatory cytokines level.

There are some proves that chronic low-grade inflammation is thought to be keyin the pathogenesis of insulin resistance, type 2 diabetesmellitus (T2DM) and cardiovascular disease that isassociated with obesity-mediated diabetes (Ouchi N, 2011). Circulating levels of TNF-a and IL-6 are directly correlated with adiposity and insulin resistance (YueP, 2011). Other way, IL-6 increases glucose stimulated insulin secretion (Suzuki T, 2011).

According to obtained data we also analyzed amount patients with dysglicemia in each cluster (see fig. 3). So, 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 3rd and 4th clusters had hypertension and comorbid carbohydrate pool abnormalities in 85,6% and 91,8% correspondingly.

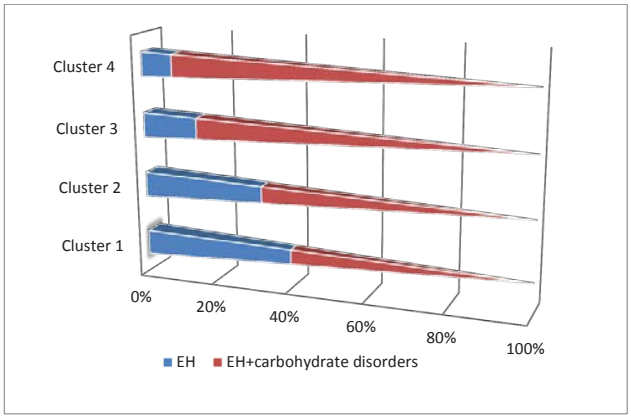


**Fig. 3. Essential hypertension and percentage of accompanied dysglycemia in clusters.**

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, $p<0,05$).

It's shown that apelin has effects not only on glucose utilization, but also apelin's receptor is expressed in islets and apelin activation of its receptor inhibits insulin secretion. And it has been shown in clonal INS-1 β-cells that this is by activation of PI3K0phosphodiesterase 3B (GuoL,2009). Recent evidence suggests that apelin is itself expressed in

Table 1

**Anamnesis data, results of laboratory and instrumental investigations of patients with essential hypertension according to clusters**

| Groups / Means | Cluster 1, 23 pts with EH | Cluster 2, 22 pts with EH | Cluster 3, 14 pts with EH | Cluster 4, 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/m$^2$ | 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 |
| 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 |
| 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 |
| IL-10, pg/ml | 80,56 (76,50; 88,60) | 90,45 (79,50; 91,60) | 78,84 (74,85; 83,80) | 88,30 (78,74; 90,60) | 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 |

*Data is described by median and inter-quartile range.*

*Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, totalcholesterol; 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; IL-6, interleukine-6; IL-10, interleukine-10.*

pancreatic islets, particularly in β- and α-cells, raising the possibility of autocrine/paracrine effects (Ringstroem C, 2010).

So, our study shows the increased level of peptide apelinin hypertensive patients. Obesity is not always associated with expression of adipokine, but depends from pronunciation of accompanied dyslipidemia and carbohydrate metabolism disturbances. Significant dyslipidemia with high atherogene index,dysglicemia, hyperinsulinemia, and pronounced expression of pro-inflammatory cytokine are accompanied with decreasing of apelin level and negative correlation of BMI with peptide. Overexpression of apelin in hypertensive patients with moderate abnormalities in lipid and carbohydrate metabolism is considered as compensatory reaction. Further investigations of apelin activity will lead to clarifying the potential links of metabolic parameters with peptide expression.

**Conclusion:**

1. Plasma level of adipokine apelin is increased in patients with essential hypertension and obesity.
2. Obesity is associated with expression of adipokine and accompanied with dyslipidemia and carbohydrate metabolism disturbances.
3. Pronounced pro-inflammatory state, dyslipidemia with high atherogene index, dysglicemia, hyperinsulinemia in patients with essential hypertension and obesity are accompanied with decreasing of apelin level and negative correlation of BMI with peptide.
4. Overexpression of apelin in hypertensive patients with moderate abnormalities in lipid and carbohydrate metabolism is considered as compensatory reaction.



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## ДОСЛІДЖЕННЯ АКТИВНОСТІ АПЕЛІНА У ХВОРИХ НА ГІПЕРТОНІЧНУ ХВОРОБУ З ОЖИРІННЯМ

**Анотація**
Асоціація гіпертонічної хвороби й ожиріння є полікомпонентним ланцюгом метаболічних порушень. Причини розвитку ожиріння у хворих на гіпертонічну хворобу (ГХ) й досі не з'ясовані. Хворі розподілені на кластери із використанням к-середніх за ІМТ та апеліном. Встановлено підвищення рівня апеліну у хворих на ГХ у порівнянні з групою контролю. Виразна дисліпідемія з підвищеним індексом атерогенності, гіперінсулінемія, гіперцитокінемія супроводжувалась зниженням рівня апеліну й негативним взаємозв'язком пептиду з ІМТ.
**Ключові слова:** гіпертонічна хвороба, ожиріння, адипокіни, апелін, дисліпідемія.

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## ИССЛЕДОВАНИЕ АКТИВНОСТИ АПЕЛИНА У БОЛЬНЫХ ГИПЕРТОНИЧЕСКОЙ БОЛЕЗНЬЮ С ОЖИРЕНИЕМ

**Аннотация**
Ассоциация гипертонической болезни и ожирения представляет собой многокомпонентную цепь метаболических нарушений. Причины развития ожирения у больных с гипертонической болезнью (ГБ) до конца не известны.
Пациенты были разделены на кластеры с использованием к-средних по ИМТ и апелину. Установлено повышение уровня апелина у больных гипертонической болезнью в сравнении с группой контроля. Выраженная дислипидемия с повышенным индексом атерогенности, гипринсулинемия, гиперцитокинемия сопровождалась снижением уровня апелина и отрицательной взаимосвязью пептида с ИМТ.
**Ключевые слова:** гипертоническая болезнь, ожирение, адипокины, апелин, дислипидемия.