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PLATELETS AMINO ACIDS PROFILE AND CARDIOMETABOLIC RISK FACTORS IN PATIENTS WITH CORONARY ARTERY DISEASE AND ATRIAL FIBRILLATION

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The aim: to found out connections between platelets amino acids profile and cardiometabolic risk factors in coronary artery disease patients with atrial fibrillation. 300 patients were divided into 3 groups: first (I) – 149 patients with coronary artery disease and without arrhythmias, second (II) – 123 patients with coronary artery disease and atrial fibrillation paroxysm and 28 patients in control group. In II group in comparison with I group were checked (P<0.05): increasing isoleucine (10.73 %), leucine (12.63 %) and decreasing threonine (23.05 %), serine (5.06 %), glycine (32.21 %), valine (30.83 %) levels in platelets amino acids profile; elevation of apolipoprotein B (29.91 %), C-reactive protein (40.93 %), interleucine-6 (22.93 %), trimethylamine (16.13 %) and trimethylamine-N-oxide (57.54 %) levels and fall of trimethylamine / trimethylamine-N-oxide ratio (26.16 %). Majority of correlations were found between glycine (total number = 12), threonine (total number = 6), glutamate (total number = 6), valine (total number = 6) and cardiometabolic risk factors. Platelets glycine correlated with age (r=-0.305), body mass index (r=-0.351), total cholesterol (r=-0.304), low density lipoprotein (r=-0.348), apolipoprotein A1 (r=0.373), apolipoprotein B (r=-0.347), interleucine-6 (r=-0.315), trimethylamine-N-oxide (r=-0.654), trimethylamine / trimethylamine-N-oxide ratio (r=0.688), prothrombin index (r=0.317), activated partial thromboplastin time (r=-0.365) and fibrinogen (r=-0.396), P<0.05.

Key words: coronary artery disease, atrial fibrillation, amino acids, blood platelets, cardiometabolic risk factors.

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АМІНОКИСЛОТНИЙ ПРОФІЛЬ ТРОМБОЦИТІВ ТА КАРДІОМЕТАБОЛІЧНІ ФАКТОРИ РИЗИКУ У ХВОРИХ НА ІШЕМІЧНУ ХВОРОБУ СЕРЦЯ ТА ФІБРИЛЯЦІЮ ПЕРЕДСЕРДЬ

Мета: виявити зв’язок між амінокислотним профілем тромбоцитів і кардіометаболічними факторами ризику у пацієнтів з ішемічною хворобою серця та фібриляцією передсердь. 300 пацієнтів були розподілені на 3 групи: перша (I) – 149 пацієнтів з ішемічною хворобою серця та без аритмій, друга (II) – 123 пацієнти з ішемічною хворобою серця та пароксизмом фібриляції передсердь та 28 пацієнтів контрольної групи. У II групі порівняно з I групою виявлено (P<0,05): підвищення ізолейцину (10,73 %), лейцину (12,63 %) та зниження рівнів треоніну (23,05 %), серину (5,06 %), гліцину (32,21 %), валіну (30,83 %) у амінокислотному профілі тромбоцитів; підвищення рівнів аполіпопротеїну В (29,91 %), С-реактивного білка (40,93 %), інтерлейкіну-6 (22,93 %), триметиламіну (16,13 %) і триметиламін-N-оксиду (57,54 %) і зниження співвідношення триметиламін / триметиламін-N-оксид (26,16 %). Більшість кореляцій виявлено між гліцином (загальна кількість = 12), треоніном (загальна кількість = 6), глутаматом (загальна кількість = 6), валіном (загальна кількість = 6) і кардіометаболічними факторами ризику. Рівень гліцину в тромбоцитах корелює з віком (r=-0,305), індексом маси тіла (r=-0,351), холестерином (r=-0,304), ліпопротеїдами низької щільності (r=-0,348), аполіпопротеїном А1 (r=0,373), аполіпопротеїном В (r=-0,347), інтерлейкіном-6 (r=-0,315), триметиламін-N-оксидом (r=-0,654), співвідношенням триметиламін / триметиламін-N-оксид (r=0,688), протромбіновим індексом (r=0,317), активованим частковим тромбопластиновим часом (r=-0,365) та фібриногеном (r=-0,396), P<0,05.

Ключові слова: ішемічна хвороба серця, фібриляція передсердь, амінокислоти, тромбоцити, кардіометаболічні фактори ризику.

The study is a fragment of the research project “Changes in protein, carbohydrate and lipid metabolism in patients with coronary heart disease and arterial hypertension with heart rhythm disorders, possibilities of drug correction”, state registration No. 0121U108875.

Atrial fibrillation (AF) is the most widely spread arrhythmia all over the world. It is connected with increasing mortality and hospitalizations quantity. Stroke, heart failure, dementia, depression and impaired life quality are the well-known AF complications. AF presence is directly connected with thrombosis risk and anticoagulation treatment is the one of the basic in AF management. Coronary artery (CAD) is the most common cardiovascular disorder, that is also characterized by prothrombotic state. Moreover, CAD is an independent AF risk factor and vice versa: together they both worsening course of each other [3, 6, 10].

Amino acids (AA) play the crucial role in platelets activation. Branched chain AA (BCAA) have a prothrombotic properties by increasing levels trombomodulin-3 and integrin αIIbβ3. Valine catabolite α-ketoisovaleric acid has the strong promotion effect on platelets activation [13]. At the same time taurine and glycine have antithrombotic potential. Glycine can block the calcium flow into platelets by the hyperpolarization of platelets membrane chlorine channels. Taurine decline platelets activation by inhibition platelets derived growth factor BB [9]. Platelets amino acids spectrum in patients with AF are still uninvestigated, whereas it is changed for some metabolic diseases, as diabetes mellitus, etc., which are known AF risk factors [3].

Factors, which contribute to cardiovascular diseases and diabetes are called cardiometabolic risk factors (CMRF). Obesity, hyperglycemia, hypercholesterolemia, hypertriglyceridemia, rise of low-density lipoproteins (LDL), apolipoprotein B (ApoB), lipoprotein α (Lpα), inflammatory markers as C-reactive protein (CRP) and interleukin-6 (IL-6) levels, decrease of high-density lipoproteins (HDL), apolipoprotein A1 (ApoA1) are well known CMRF [5, 7]. Role of the gut microbiota metabolites in cardiovascular diseases pathogenesis is still under discussion. According to the latest data trimethylamine (TMA) and trimethylamine-N-oxide (TMAO) levels are directly connected with CAD and AF development [8].

Thus, platelets AA profile and its connections with known CMRF is an interesting pathogenetic question in AF paroxysm pathogenesis in CAD patients. That can help us to find out the new biochemical risk factors of AF development in CAD patients.

The purpose of the study was to found out connections between platelets amino acids profile and cardiometabolic risk factors in coronary artery disease patients with atrial fibrillation.

Materials and methods. 300 patients were included in the study and were divided into 3 groups: first (I) – 149 patients with CAD and without arrhythmias, second (II) – 123 patients with CAD and AF paroxysm and control group (CG) – 28 patients without CAD and arrhythmias. CAD and AF diagnosis were based on the latest ESC guidelines [3, 6]. Diagnosis CAD was confirmed by the history of coronary arteries stenotic changes during invasive coronarography. AF paroxysm was checked by resting 12 leads electrocardiography. Exclusion criteria were: reported malignancies, chronic kidney disease (Glomerular Filtration Rate, GFR <60 mL/min), valvular AF, heart failure Class III to IV (by New York Heart Association), thyroid pathology, inflammatory bowel disease, irritable bowel syndrome, vegetarians and vegans, pregnancy, taking probiotics and antibiotics for a month before the study. No significant difference in risk factors at baseline were seen between investigated groups. The study was conducted at the base and was approved by the Ethical commission of the Kiev City Clinical Hospital No. 12. Informed consent was obtained from all subjects in accordance with the Declaration of Helsinki. Baseline characteristics of the study sample are shown in Table 1.

Table 1

Baseline characteristics of the study sample, mean ± standard error

Characteristic /group	I	II	CG	P1-2	P2-3	P1-3
Age (years)	67.71±3.90	67.96±0.94	56.25±2.18	P>0.05	P>0.05	P>0.05
Men (%)	48.99	47.97	48.15	P>0.05	P>0.05	P>0.05
BMI (kg/m^2)	27.02±0.33	26.93±0.43	28.12±2.10	P>0.05	P>0.05	P>0.05
Uric acid (mmol/l)	380.5±28.16	404.9±36.11	310.2±29.12	P>0.05	P<0.05	P<0.05
Total bilirubin (mmol/l)	11.3±0.09	12.4±0.08	11.7±0.11	P>0.05	P>0.05	P>0.05
GFR (ml/min)	62.03±2.31	67.73±1.98	84.01±5.48	P>0.05	P<0.05	P<0.05
PI, %	82.10±0.78	77.16±0.69	81.08±1.82	P>0.05	P<0.05	P>0.05
Ht, %	43.93±0.48	45.17±0.64	43.08±1.05	P>0.05	P>0.05	P>0.05
aPTT, s	29.33±0.40	30.29±0.62	27.30±0.56	P>0.05	P<0.05	P>0.05
Fibrinogen, mg/dl	2.89±0.08	3.21±0.09	2.65±0.11	P<0.05	P<0.05	P>0.05
Fibrin, mg	17.26±0.46	16.73±0.47	17.54±1.81	P>0.05	P>0.05	P>0.05

Platelets AA level was detected by method of ion exchange liquid column chromatography. There were identified AA: lysine, histidine, arginine, ornithine, taurine, asparagine acid, threonine, serine, glutamine acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, glutamine, ammonia. Blood sampling from patients was performed on an empty stomach from the cubital vein on the first day of hospitalization, before treatment. Citrated blood was centrifuged for 10 minutes at a speed of 1500 revolutions per minute. The middle layer is selected with a Pasteur pipette - the plasma was saturated with platelets. The obtained material was again centrifuged for 20 minutes at a speed of 3000 revolutions per minute. The upper supernatant liquid was collected with a Pasteur pipette, and the lower layer was washed with buffer (pH 6.2). Washed platelets are resuspended in buffer (pH 7.4).

We studied such cardiometabolic risk factors: total cholesterol (TC), triglycerides (TG), LDL, HDL, Lpα, apolipoprotein A1 (ApoA1), ApoB, CRP, IL-6, TMA and TMAO. Also, ApoB/ApoA1 and TMA/TMAO ratios were checked. The level of TMAO, TMA plasma was determined by gas chromatography with mass electron detection. They were extracted from blood plasma into acid by adding internal standards. Patient's blood sampling was performed on an empty stomach from the cubital vein on the day of hospitalization. Hymalyzer 2000 was used for detection TC, TG, HDL, LDL (reagent produced by HUMAN GmbH), ApoA1, ApoB, Lpα and CRP (reagent produced by Dialab) – by flow cytometry. Hymareader 2106 (ELISA) was used for detection IL-6 – reagents produced by Vector Best.

Results were presented as mean ± standard error for continuous variables. Variables distribution for normality were checked by the Pearson criterion. Data were compared by Scheffe's or Dann multiple comparison methods depends on two critical regions for variables distribution respectively; Spearman's rank correlation coefficient was detected [2]. All calculations were done in MATLAB R2014a (License number 271828).

Results of the study and their discussion. In our study, we investigated platelets AA spectrum in CAD patients with AF. Results are shown in Fig. 1.

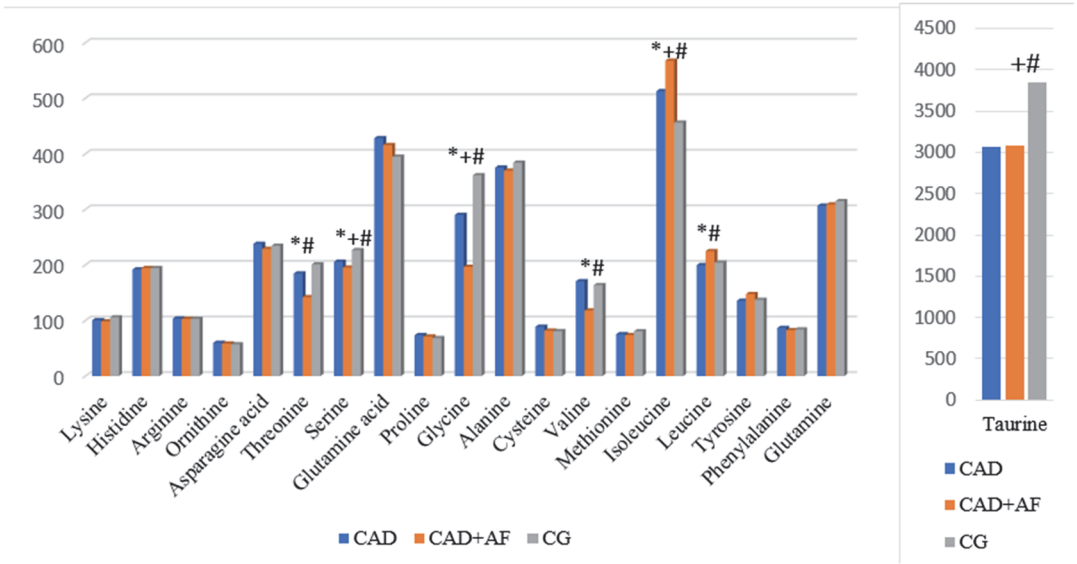

Fig. 1. Platelets amino acids profile in investigated groups, mkmol/l. Notes: * – P<0.05 I-II groups; + – P<0.05 group I– CG; # – P<0.05 group II– CG.

For patients with CAD and without arrhythmias significant rise of isoleucine (12.41 %) and decline of taurine (20.26 %), serine (9.31 %) and glycine (19.73 %) levels were revealed in comparison with CG. For patients with CAD and AF were found significant elevation of isoleucine (24.47 %), leucine (10.20 %) and descent of taurine (19.84 %), threonine (29.37 %), serine (13.90 %), glycine (45.59 %) and valine (27.87 %) levels in comparison with CG. Moreover, in CAD patients with AF in comparison with CAD patients without arrhythmias significant increasing isoleucine (10.73 %), leucine (12.63 %) and decreasing threonine (23.05 %), serine (5.06 %), glycine (32.21 %), valine (30.83 %) levels were checked.

CMRF were studied in CAD patients with AF. Results are shown in Fig. 2.

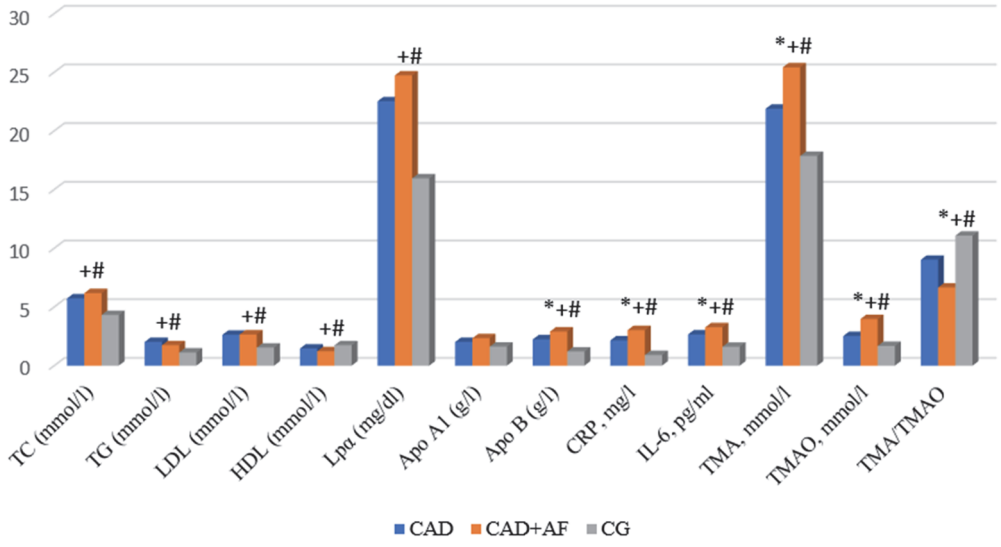

Fig. 2. Cardiometabolic risk factors of investigated groups

Notes: * – P<0.05 I-II groups; + – P<0.05 group I– CG; # – P<0.05 group II– CG.

A significant rise of TC (32.64 % and 43.06 % respectively), TG (80.36 % and 55.36 % respectively), LDL (70.78 % and 72.73 % respectively), Lpα (41.17 % and 54.95 % respectively), ApoB (85.12 % and 140.50 % respectively), CRP (136.26 % and 232.97 % respectively), IL-6 (65.22 % and 103.11 % respectively), TMA (22.50 % and 42.25 % respectively), TMAO (50.00 % and 136.31 %

respectively) and decline of HDL (16.09 % and 29.31 % respectively), TMA/TMAO ratio (18.59 % and 39.89 % respectively) were found in CAD patient with and without AF compared with CG (P<0.05). Significant elevation of ApoB (29.91 %), CRP (40.93 %), IL-6 (22.93 %), TMA (16.13 %), TMAO (57.54 %) levels and fall of TMA/TMAO ratio (26.16 %) were revealed in CAD patients with AF in comparison with CAD patients without arrhythmia.

The correlation analysis between platelets AA profile and cardiometabolic risk factors was done in investigated groups. All correlations are shown in the Fig. 3.

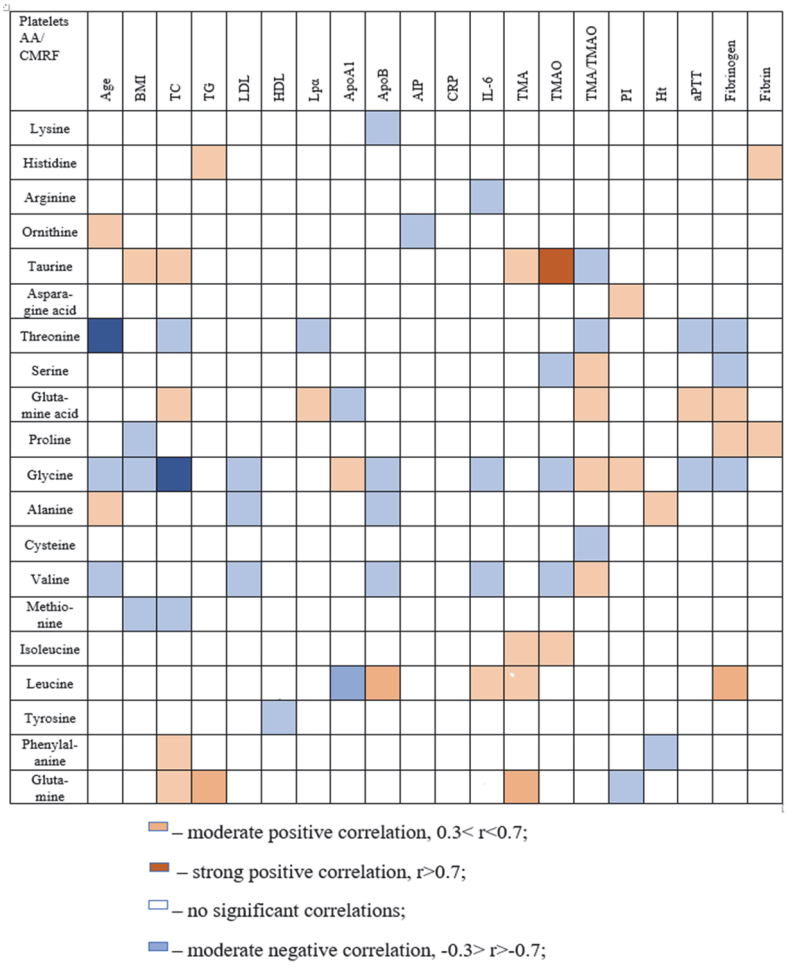

Fig. 3. Correlations heatmap between platelets amino acids and cardiometabolic risk factors, P<0.05

A lot of correlations was checked between platelets AA profile and such characteristics as TMA/TMAO (total number = 7), TC (total number = 7) and fibrinogen (total number = 6) levels. The main correlations were found between glycine (total number = 13), threonine (total number = 6), glutamate (total number = 6), valine (total number = 6) and CMRF.

Significant elevation of ApoB, CRP, IL-6, TMA, TMAO levels and fall of TMA/TMAO ratio were revealed in CAD patients with AF in comparison with CAD patients without arrhythmia. This data is matched with the results of different previous studies [5, 7, 8].

Significant BCAA platelets spectrum changes in CAF patient with AF were found in our study: rise of isoleucine, leucine and decline of valine levels. Valine is an important resource for valine/α-ketoisovaleric acid metabolic pathway that plays an important way in platelets activation [13]. Moreover, BCAA promotes megakaryocytes differentiation and platelets biogenesis [4]. Leucine, isoleucine and valine can have different metabolic effects. For example, in lipogenesis valine decrease odd-chain fatty acids level, that associated with lower risk of diabetes mellitus type 2. At the same time, leucine provides fatty acids transportation and regulate their food intake. Also, leucine can promote platelets activation through mTOR signaling pathway by rise of S6 ribosomal protein phosphorylation [1]. Leucine and isoleucine increasing are associated with cardiac fibrosis development, which is one of the important pathogenetic mechanism of AF paroxysm development [12].

On the other hand, diminution of threonine, serine and glycine levels in platelets AA profile in CAD with AF patients were present according to our results. Biochemically threonine, glycine and serine metabolism are closely linked. Threonine and glycine are also taking part in mTOR metabolic pathway regulation through protein synthesis modulation. Moreover, threonine stimulates anti-inflammatory cytokines production, modulates gut microbiota condition and normalize intrahepatic triglycerides metabolism [11]. Glycine inhibits platelets aggregation through the activation of glycine-gated chloride channels in platelets, that hyperpolarize platelets membrane and lead for calcium channels blockage. Serine metabolism is also promoting intrahepatic lipogenesis, decrease triglycerides production, have anti-inflammatory action [14].

The role of glycine, threonine and valine exchange in cardiometabolic risks formation is shown by the huge amount of significant correlations between known CMRF and platelets glycine, threonine and valine levels.

Conclusions

Significant connections between platelets amino acids profile and cardiometabolic risk factors in coronary artery disease patients with atrial fibrillation were investigated in our study. Results that were obtained:

1. Significant increasing isoleucine (10.73 %), leucine (12.63 %) and decreasing threonine (23.05 %), serine (5.06 %), glycine (32.21 %), valine (30.83 %) levels in platelets amino acids profile were checked in coronary artery disease patients with atrial fibrillation in comparison with coronary artery disease patients without arrhythmias, $P<0.05$.

2. Significant elevation of apolipoprotein B (29.91 %), C-reactive protein (40.93 %), interleucine-6 (22.93 %), trimethylamine (16.13 %) and trimethylamine-N-oxide (57.54 %) levels and fall of trimethylamine/trimethylamine-N-oxide ratio (26.16 %) were revealed in coronary artery disease patients with atrial fibrillation in comparison with coronary artery disease patients without arrhythmia, $P<0.05$.

3. A lot of correlations were checked between platelets amino acids profile and trimethylamine/trimethylamine-N-oxide ratio (total number = 7), total cholesterol (total number = 7) and fibrinogen (total number = 6) levels. Besides, majority correlations were found between glycine (total number = 12), threonine (total number = 6), glutamate (total number = 6), valine (total number = 6) and cardiometabolic risk factors.

4. Platelets glycine was significantly correlated with the majority of cardiometabolic risk factors: age ($r=-0.305$), BMI ($r=-0.351$), total cholesterol ($r=-0.304$), low-density lipoproteins ($r=-0.348$), apolipoprotein A1 ($r=0.373$), apolipoprotein B ($r=-0.347$), interleucine-6 ($r=-0.315$), trimethylamine-N-oxide ($r=-0.654$), trimethylamine/trimethylamine-N-oxide ratio ($r=0.688$), prothrombin index ($r=0.317$), activated partial tromboplastin time ($r=-0.365$) and fibrinogen ($r=-0.396$), $P<0.05$.

Prospects of further research. Platelets amino acids profile and cardiometabolic risk factors correction will be interesting approach for further investigations.

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