METABOLIC AND HEMOSTATIC PARAMETERS IN PRE-DIABETES AND NEWLY DIAGNOSED TYPE 2 DIABETES

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Metabolic changes in diabetes mellitus are associated with hemostatic disorders. However, the sequence of hemostatic events in the early stages of disease remains unclear.

Aim. To assess metabolic and hemostatic parameters and their interaction in pre-diabetes and newly diagnosed type 2 diabetes mellitus (ND T2DM).

Material and methods. The study enrolled volunteers of 40 to 65 years who considered themselves healthy and did not get any medication therapy. Of 170 examined individuals, 46 had impaired carbohydrate exchange (ICE) — 13 with impaired fasting glucose, 17 — impaired glucose tolerance and 16 with ND T2DM. The control group comprised healthy volunteers with normal body mass index and without signs of metabolic abnormalities. The metabolic (carbohydrate, lipid, protein exchange, hepatic transaminase), platelet and plasma hemostatic parameters (mean platelet volume, ADP- &collagen-induced platelet aggregation, coagulation profile, fibrinogen, plasminogen) were investigated. We identified the peculiarities initiating impact of changed parameters on different hemostatic components in patients with pre-diabetes and ND T2DM.

Results. Concentration of insulin, C-peptide, Homa-IR, total cholesterol demonstrated increase in groups with ICE. ADP-induced platelet aggregation, fibrinogen increased in ICE, however these changes were not statistically significant. Mean platelet volume and plasminogen had the tendency to be elevated in pre-diabetes and demonstrated significant increase in ND T2DM.

Conclusion. Metabolic disorders in prediabetic stage initiate changes in platelet hemostasis and fibrinolysis. The increase of MPV and higher concentrations of plasminogen are considered to be significant in ND T2DM.

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Key words: pre-diabetes, newly diagnosed type 2 diabetes mellitus, metabolic disorders, hemostasis — platelets, coagulation, fibrinolysis.

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MPV — mean platelet volume, FPG — fasting plasma glucose, APPT — activated partial thromboplastin time, HDL-CL — high-density lipoprotein-cholesterol, LDL-CL — low-density lipoprotein-cholesterol, TG — triglyceride, HOMA-IR — homeostasis model assessment-estimated insulin resistance, C-p — C-peptides, HbA_{1c} — hemoglobin A1c, BMI — body mass index, WC — waist circumference, C-p — C-peptides, IR — insulin resistance, TC — total cholesterol, AST — aspartate transaminase, ALT — alanine transaminase, ADP — adenosine diphosphate, PLG — plasminogen.

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ПАРАМЕТРЫ МЕТАБОЛИЗМА И ГЕМОСТАЗА ПРИ ПРЕДИАБЕТЕ И ВПЕРВЫЕ ВЫЯВЛЕННОМ САХАРНОМ ДИАБЕТЕ 2 ТИПА

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Метаболические изменения при сахарном диабете (СД) сопряжены с нарушениями гемостаза, последовательность вовлечения компонентов которого на ранних стадиях заболевания не определена.

Цель. Изучение параметров метаболизма, гемостаза и характера их взаимоотношений при предиабете и впервые выявленном СД 2 типа (ВВ СД2).

Материал и методы. Объектом исследования явились добровольцы в возрасте 40-65 лет считающие себя здоровыми и не получающие никакой медикаментозной терапии. У 46 из 170 обследованных выявлены нарушения углеводного обмена (НУО): 13 — нарушение гликемии натощак,17 нарушение толерантности к глюкозе и у 16 ВВ СД2. Контрольную группу составили 13 здоровых добровольцев с нормальной массой тела без признаков метаболически аномального фенотипа. Во всех контингентах исследованы параметры метаболизма (углеводный, липидный, белковый обмен, трансаминазы печени) и тромбоцитарно-плазменного гемостаза (средний объем тромбоцитов, ADP- и коллаген-индуцированная агрегация тромбоцитов, коагулограмма, фибриноген, плазминоген). Определены особенности инициирующих влияний измененных параметров метаболизма на различные компоненты гемостаза при предиабете и ВВ СД2.

Результаты. Концентрация инсулина, С-пептида, HOMA-IR, общее содержание холестерина были повышены в группах с нарушением углеводного обмена. АДФ и коллагеном-вызванной агрегацию тромбоцитов, фибриноген так же были повышены при НУО, однако эти изменения не были статистически значимы. Средний объем тромбоцитов и плазминогена имели склонность к увеличению на стадии предиабета и продемонстрировали статистически значимое увеличение при ВВ СД2.

Заключение. Метаболические нарушения на стадии предиабета инициируют изменения тромбоцитарного гемостаза и фибринолиза. При ВВ СД2 увеличение MPV и повышение концентрации плазминогена принимают значимый характер.

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Ключевые слова: предиабет, впервые выявленный сахарный диабет 2 типа, метаболические нарушения, гемостаз — тромбоцитарный, коагуляционный, фибринолиз.

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Type 2 diabetes mellitus (T2DM) is a significant risk factor for cardiovascular disease [1]. Debuting as a metabolic disease T2DM is accompanied by morphologic changes of the vascular wall, target-organ damaging, developing cardiovascular catastrophes. Atherothrombosis is the leading cause of death in T2DM. People with DM are two times more likely to have a heart disease or stroke than people who do not [2]. It is therefore particularly interesting to

study how the changed metabolic parameters influence on hemostasis. Many investigations and reviews on this issue represent an amazingly deep biochemical analysis of specific triggers of endothelial cell damage, dysfunction of platelets, plasmatic hemostasis [3-5].

However, we didn't find any available reports, describing and identifying connection between separate hemostatic and metabolic parameters of early stages of DM2 on one representative clinical material. Therefore in the present study we investigated metabolic and hemostastic parameters and their relationships in patients with pre-diabetes and with newly diagnosed type 2 diabetes mellitus (ND T2DM).

Material and methods

This study enrolled volunteers aged 40 to 65, who were non-smokers, considered themselves healthy and did not get any medication therapy. Of 170 examined individuals, 46 had impaired carbohydrate exchange (ICE) and met the inclusion criteria: ND T2DM, impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). Exclusion criterion was the presence of any other disease, including the previously identified diabetes and the presence of diabetic micro- and macrovascular complications.

DM2 diagnosis is based on fasting plasma glucose level (FPG) ≥7,0 mmol/l (126 mg/dl) or 2–h plasma glucose ≥11,1 mmol/l (200 mg/dl) and HbA_{1c} >6,5%. If level of fasting plasma glucose was 6,1 — 6,9 mmol/L (110 mg/dl to 125 mg/dl) and/or HbA_{1c} 5,7-6,4%, oral glucose tolerance test (OGTT) (75 g oral glucose load) was done. Based on its results, pre-diabetes state was classified as — IFG [(fasting blood glucose 6,1–6,9 mmol/L (110 mg/dl to 125 mg/dl) and 2–h plasma glucose <7,8 mmol/l (140 mg/dl)] or IGT [(fasting plasmaglucose 6,1–6,9 mmol/L (110 mg/dl to 125 mg/dl) and 2-h glucose ≥7,8 and <11,1 mmol/l (140 mg/dl and 200 mg/dl) [6-8].

Systolic and diastolic blood pressure (SBP and DBP, respectively) was measured according to the standard technique [9]. The study included patients with SBD <140 mmHg and DBD <90 mmHg.

The height, weight, waist and hip circumferences were measured in underwear and without shoes. Body mass index (BMI) was calculated as weight divided by squared height (kg/m^2).

The control group comprised non-smoking healthy volunteers with normal body mass index (BMI 18,5-24,9 kg/m^2, waist circumference (WC): women <88 cm, men <102 cm, without signs of metabolic abnormalities (BP <130/85 mmHg, fasting glucose <5,5 mmol/l, triglycerides <1,7 mmol/l, HDL cholesterol >1,04 mmol/l in men, and >1,3 mmol/l in women, HOMA IR <2,5).

The Ethical Committee of Kuban State Medical University, Krasnodar, approved the study and all patients and controls gave written informed consent.

Laboratory Research. To estimate the parameters of metabolism and hemostasis venous blood samples was performed after a 12-hour overnight fasting, venous blood samples were drawn in the morning (between 8:00 AM and 9:00 AM).

The hemogram was evaluated using the Automatic Hematology Analyzer ADVIA 120 (Siemens, Germany); the biomedical parameters (including total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST)) were determined using enzymatic colorimetric method with the Automatic Biochemistry Analyzer ADVIA 1650, 2400 (Siemens, Healthcare Diagnostics, USA).

Glycated hemoglobin (HbA1c) was measured using reference method (HPLC D-10) (Bio-Rad Laboratories, USA), calibrated to the Diabetes Control and Complications Trial standard. Determination of insulin and C-peptide was performed on an automated immunochemical analyzer Advia Centaur, manufactured by Siemens Healthcare Diagnostics, USA. Homeostasis Model Assessment Model (HOMA) was used to determine the insulin sensitivity index with formula: HOMA-IR=fasting insulin (μU/ml) x fasting glucose (mmol/l)/22,5.

Serum protein fractions were separated by zone electrophoresis on an agarose gel using an automated electrophoresis system Hydrasys (Sebia, France).

All hemostasis-related parameters were determined by turbidimetric and chromogenic methods on the ACL TOP 700 Automated Analyzer (Instrumentation Laboratory Company, USA) using anufacturer`s reagent kit.

Platelet aggregation activity was examined on a turbidimetric platelet aggregation laser-analyzer "Biola 230 LA" (Russia). Sodium adenosintriphosphate (LLC Technology-standard, Russia) and collagen (SPA Renam, Russia) were used as inductors of platelet aggregation in both the cases at the final concentration of 30 μM.

Statistical analysis was performed using the package of program STATISTICA 10 (StatSoft, USA). The results are expressed as Median, upper and lower quartiles (Me (25; 75), where Me — median, 25 and 75 are the 1st and 3d quartiles) comparing the mean ranks for all groups using the Kruskal-Wallis test. Spearman's rank correlation test was used to reveal the correlation between the indexes equal to P-value=0,05 was considered statistically significant.

Results

According to the stated objectives, the patients were grouped into early diabetes (n=16), IGT (n=17) and IFG (n=13) and normal controls (n=13). The group was similar in sex and age (Table 1). However, patients with ICE had excessive BMI exceeding normal index by 50%, 41% and 44% with ND T2DM, IGT and IFG respectively. WC in groups with ICE was also significantly higher than in the normal group.

Analysis of biochemical parameters in patients with ICE showed changes of both carbohydrate and lipid profiles as

Table 1

Clinical characteristics of patients with newly diagnosed type 2 diabetes mellitus, pre-diabetes and control

	1 ND T2DM n=16	2 IGT n=17	3 IFG n=13	4 Control n=13	5 p
Age, year	57,0 (51,0; 61,5)	54,0 (49,0; 57,0)	55,0 (54,0; 56,0)	47,0 (45,0; 53,0)	p_{1-4}=0,06 p_{2-4}=0,95 p_{3-4} =0,62
Sex, M/F	13/3	15/2	10/3	11/13	
BMI, kg/m^2	34,6** (33,2; 39,9)	32,1** (26,5; 34,8)	32·8** (28,7; 33,3)	22,8 (21,2; 23,9)	p_{1-4}=0,000 p_{2-4}=0,000 p_{3-4}=0,001
Waist circumference, cm	109** (106; 113)	99** (94; 106)	102** (93; 111)	76 (72; 78)	p_{1-4}=0,000 p_{2-4}=0,003 p_{3-4}=0,001
Hip circumference, cm	115** (107; 124)	109 (101; 119)	111** (108; 118)	97 (95;103)	p_{1-4}=0,000 p_{2-4}=0,016 p_{3-4}=0,002
SBP, mmHg DBP, mmHg	125* (125;130) 80 (80; 80)	125 (120;125) 80 (80;80)	125* (120; 130) 80 (80;80)	120 (110;120) 80 (75; 80)	p_{1-4}=0,03 p_{2-4}=0,06 p_{3-4}=0,02 p_{1-4}=0,33 p_{2-4}=0,94 p_{3-4}=0,35
Fasting glucose, mmol/l	7,0** (6,5; 8,2)	5,7 (5,4;5,8)	6,0* (5,5; 6,5)	5,2 (4,8; 5,5)	p_{1-4}=0,000 p_{1-2}=0,000 p_{1-3}=0,04 p_{2-3}=1,0 p_{2-4}=0,46 P_{3-4}=0,03
HbA_{1c}, %	6,8** (6,3; 7,6)	6,1* (5,9; 6,2)	5,9 (5,3; 6,7)	5,7 (5,6; 5,9)	p_{1-4}=0,000 p_{1-2}=0,07 p_{1-3}=0,001 p_{2-3}=0,84 p_{2-4}=0,01 p_{3-4}=1,0
Insulin, mU/l	15,6** (12,0; 23,8)	14,0* (12,5;19,0)	14,6* (10,7;17,6)	8,0 (5,6; 5,9)	p_{1-4}=0,000 p_{2-4}=0,002 p_{3-4}=0,005
C-peptide, ng/ml	2,7** (2,3; 3,4)	2,0** (1,8; 2,34)	2,0* (1,5; 2,4)	1,1 (1,0; 1,3)	p_{1-4}=0,000 p_{2-4}=0,003 p_{3-4}=0,02
HOMA-IR	6,6** (3,4; 8,1)	3,5* (2,9; 4,9)	3,6* (3,0; 4,8)	1,8 (1,5; 2,3)	p_{1-4}=0,000 p_{1-2}=0,47 p_{1-3}=0,84 p_{2-4}=0,002 p_{3-4}=0,005
Total cholesterol, mmol/l	5,9* (5,3; 6,8)	5,9* (5,24; 6,7)	5,8 (4,3; 6,7)	4,8 (4,5; 5,1)	p_{1-4}=0,004 p_{2-4}=0,004 p_{3-4}=0,09
LDL cholesterol, mmol/l	3,5 (3,0; 4,2)	3,7 (3,0;4,8)	3,8 (2,5; 4,5)	2,9 (2,6; 3,2)	p_{1-4}=0,25 $p_{2-4=}$0,08 p_{3-4}=0,83
HDL cholesterol, mmol/l	1,5 (1,2; 1,7)	1,54 (1,2; 1,7)	1,5 (1,5; 1,6)	1,6 (1,4; 1,7)	p_{1-4}=1,0 p_{2-4}=1,0 p_{3-4}=1,0
Triglycerids, mmol/l	1,7** (1,3; 2,5)	1,3* (1,1; 1,6)	1,5* (0,8; 2,1)	0,7 (0,6; 0,8)	p_{1-4}=0,000 p_{2-4}=0,004 p_{3-4}=0,006
Albumins, g/l	40,2 (38,8;43,6)	40,6 (39,0;41,9)	38,8 (36,2;41,9)	42,1 (40,4;44,0)	p_{1-4}=0,69 p_{2-4}=1,01 p_{3-4}=1,9

Extension of table 1

alpha-1globulin, g/l	2,2 (1,9; 2,4)	1,9 (1,8; 2,6)	2,1 (2,0; 2,3)	1,9 (1,5; 2,2)	p_{1-4}=1,63 p_{2-4}=0,85 p_{3-4}=1,4
alpha- 2 globulin, g/L	9,6 (8,8; 9,8)	9,6 (9,1; 10,7)	9,8 (9,1; 10,3)	9,0 (8,4; 9,7)	p_{1-4}=0,14 p_{2-4}=1,61 p_{3-4}=1,49
beta-1 globulin, g/l	7,3 (6,4; 8,3)	7,6 (6,2; 8,2)	7,3 (6,5; 8,1)	7,0 (6,7; 7,5)	p_{1-4}=0,64 p_{2-4}=0,77 p_{3-4}=0,17
beta-2 globulin, g/l	3,7 (3,3; 4,7)	4,69 (3,7; 5,02)	4,3 (2,9; 4,4)	3,7 (2,2; 4,4)	p_{1-4}=0,9 p_{2-4}=2,2 p_{3-4}=1,03
γ globulin, g/l	9,7 (8,9; 11,1)	10,3 (8,7; 11,8)	11,3 (8,7; 11,8)	10,8 (8,2; 12,1)	p_{1-4}=0,44 p_{2-4}=0,29 p_{3-4}=0,36
Creatinine, mmol/l	79,4 (74,9;87,6)	81,5 (78,6;92,3)	86 (80; 97,5)	77,0 (71,5;86,6)	p_{1-4}=1,0 p_{2-4}=0,88 p_{3-4}=0,67
Bilirubi, mmol/l	10,7 (8,3; 12,5)	10,4 (8,8; 12,8)	10,1 (8,9; 12,5)	10,2 (6,8; 12,0)	p_{1-4}=1,0 p_{2-4}=1,0 p_{3-4}=1,0
AST	26,0 (20,5;44,5)	22,0 (20,0;27,0)	20,0 (19,0;27,0)	21,0 (19,0;23,0)	p_{1-4}=0,58 p_{2-4}=1,0 p_{3-4}=1,0
ALT	30* (21,0;79,5)	26,0 (18,0;31,0)	23,0 (21,0;29,0)	19,0 (14,0;24,0)	p_{1-4}=0,01 p_{2-4}=0,45 p_{3-4}=0,91

Annotation: * — p< 0,05, ** — p < 0,001.

early as in the pre-diabetes stages. Concentration of insulin, C-peptide and Homa-IR was comparable in IGT and IFG groups and by 1,8 and 2 times higher than in normal. TC in all ICE groups, TG in IGT group and in IFG group were by 22%, 86% and 114% higher than in normal respectively. Maximally expressed changes were observed in ND T2DM, where two-fold increase of insulin content was registered, C-peptide, Homa-IR and TG increased by 2,5, 3,7 and 2,4 times respectively compared to the controls. We didn't identify significant changes of HDL, LDL and protein spectrum in patients with ICE. Parameters of hepatic metabolism demonstrated 1,5 times increased ALT in ND T2DM patients; nevertheless, pre-diabetes stages demonstrated just a tendency to this increase.

Comparison of the groups did not find significant differences between biochemical parameters in patients with ICE, except for Homa-IR which was by 1,8 times higher in patients with ND T2DM, than in patients with pre-diabetes (Figure 1).

The parameters of platelet hemostasis had the tendency to the elevated mean platelet volume (MPV) in patients with pre-diabetes and its significant increase in patients with ND T2DM (Figure 2).

Moreover, we noted the increase of ADP and collagen-induced platelet aggregation. However, these changes were not statistically significant (Figure 3, 4).

The parameters of plasma hemostasis didn't change significantly, however, they tended to increase at the stages

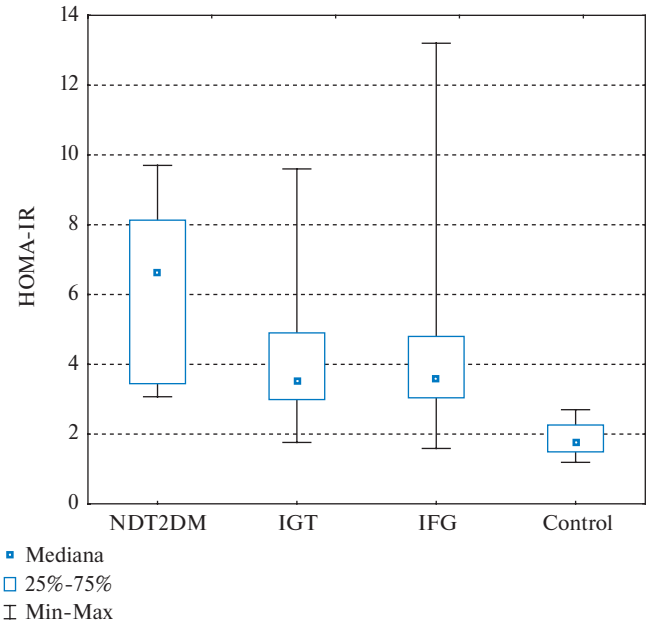

Figure 1. HOMA-IR in patients with ICE and in the control group.

of pre-diabetes and reached statistical significance in patients with ND T2DM (Figure 5).

The identified differences between the groups with regard to insulin-resistance patients with ICE, as well as their role in T2DM formation, we performed the corre-

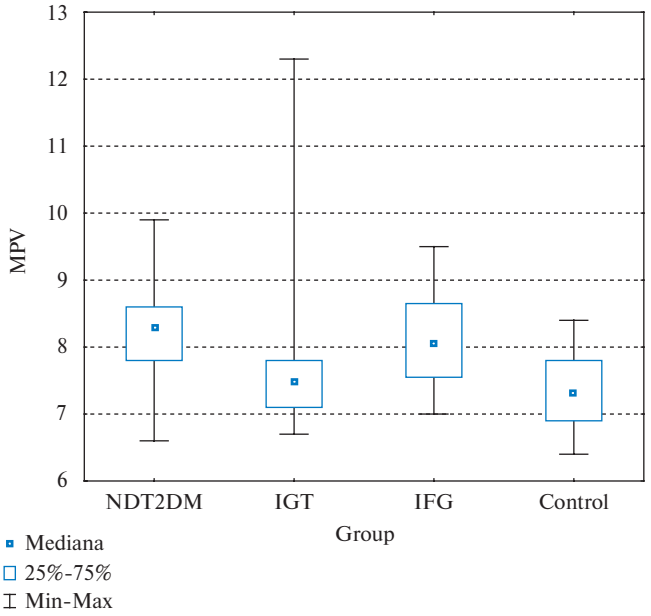

Figure 2. Mean platelet volume in patients with ICE and in the control group.

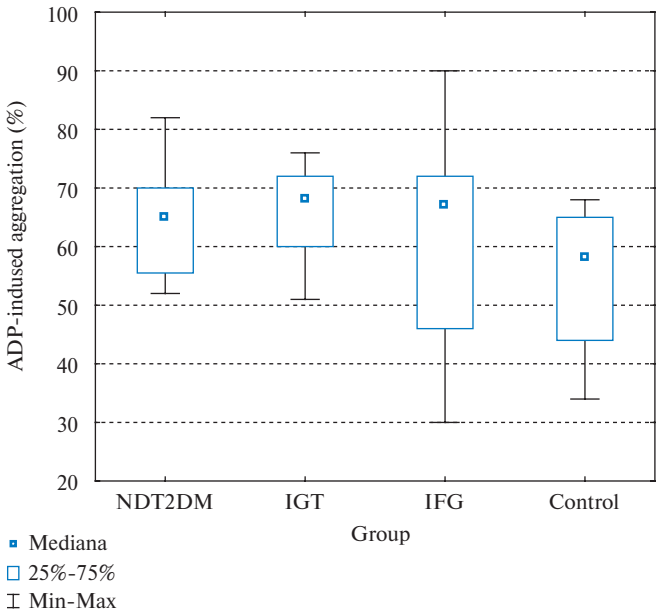

Figure 3. ADP-platelets aggregation in patients with ICE and in the control group.

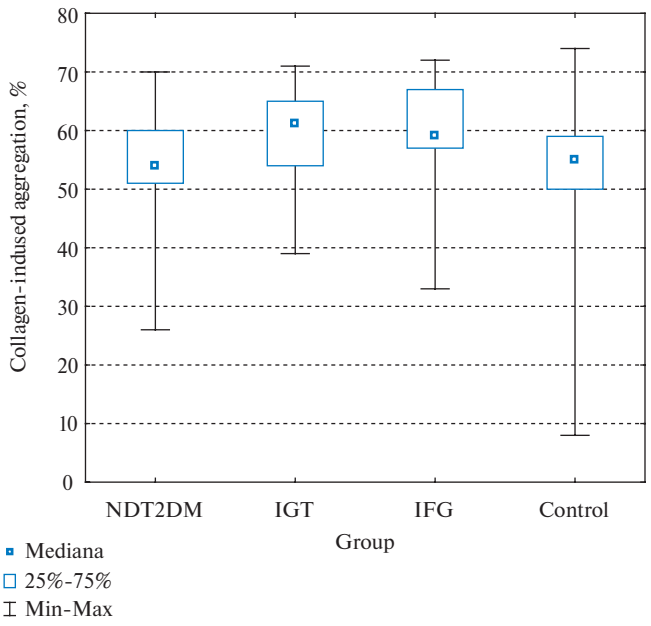

Figure 4. Collagen-indused aggregation in patients with ICE and in the control group.

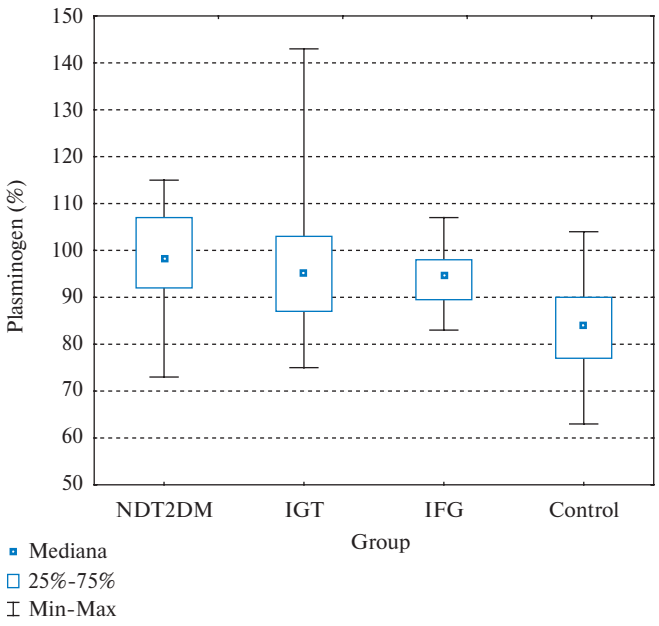

Figure 5. Plasminogen in patients with ICE in the control group.

lation analysis which revealed strong correlation between HOMA-IR and BMI, WC, fasting plasma glucose (FPG), triglycerides (Table 2), mean direct power with HOMA-IR and HbA_{1c}, TC, LDL, AST, ALT, and inverse between HOMA-IR and HDL, number of platelets. Comparing other investigated parameters of metabolism and hemostasis found that insulin had similar in character and expression of HOMA-IR correlation and weak positive connection of insulin concentration with MPV.

FPG demonstrated positive correlation of average strength with WC, HbA_{1c}, TC, LDL, TG, ALT, plasminogen, MPV and it showed inverse mathematical, but direct functional correlation with Activated Partial Thromboplastin Time (APPT). HbA_{1c} level moderately and directly correlated with BMI, WC, C-peptide, HOMA-IR, TC,TG, plasminogen, TC and TG with plasminogen, ADP-induced aggregation demonstrated positive moderate correlation with alpha 1- and beta 2- globulins and inverse correlation with albumins.

Table 2

Spearman's range correlation test

	BMI	WC	FPG	HbA_{1c}	Insulin	C-p	IR	TC	LDL	HDL	TG	AST	ALT	ADP	PLG	Platelet	MPV
FPG	0,50*	0,55*	1,00	0,54*	0,47*	0,66*	0,66*	0,46*	0,30*	-0,08	0,54*	0,17	0,33*	0,01	0,30*	-0,16	0,30*
HbA_{1c}	0,34*	0,34*	0,54*	1,00	0,23	0,39*	0,38*	0,29*	0,15	0,04	0,35*	0,05	0,22	0,15	0,35*	0,13	0,01
Insulin	0,68*	0,68*	0,47*	0,23	1,00	0,87*	0,86*	0,36*	0,30*	-0,37	0,65*	0,28*	0,50*	-0,00	0,19	-0,35*	0,28*
C-peptide	0,73*	0,77*	0,66*	0,39*	0,87*	1,00	0,88	0,43*	0,33*	-0,34	0,75*	0,31*	0,55*	0,03	0,30*	-0,31*	0,23
HOMA-IR	0,63*	0,67*	0,66*	0,38*	0,86*	0,88*	1,00	0,39*	0,31*	-0,27	0,64*	0,34*	0,47*	0,08	0,19	-0,31*	0,21
TC	0,26	0,39*	0,46*	0,29*	0,36*	0,43*	0,39*	1,00	0,91*	0,03	0,45*	0,05	0,21	0,10	0,28*	-0,13	0,06
LDL-CL	0,13	0,30*	0,30*	0,15	0,30*	0,33*	0,31*	0,91*	1,00	-0,16	0,30*	0,01	0,14	0,08	0,23	-0,14	-0,04
HDL-CL	-0,21	-0,36*	-0,08	0,04	-0,37*	-0,34*	-0,27*	0,03	-0,16	1,00	-0,38*	-0,10	-0,14	0,00	-0,06	0,15	0,03
TG	0,62*	0,73*	0,54*	0,35*	0,65*	0,75*	0,64*	0,45*	0,30*	-0,38*	1,00	0,18	0,45*	0,10	0,31*	-0,29*	0,24
Albuming/ L	-0,17	-0,26	0,04	0,01	0,03	-0,01	0,03	-0,13	-0,15	0,18	-0,22	0,22	0,13	-0,32*	-0,01	0,18	-0,19
alpha-1	0,13	0,02	0,15	0,11	-0,13	-0,04	0,05	-0,01	-0,04	0,03	0,03	-0,03	-0,11	0,45*	-0,26	0,02	-0,00
alpha-2	-0,01	-0,01	0,03	0,20	-0,24	-0,14	-0,13	0,27	0,15	0,44*	-0,06	-0,23	-0,25	0,20	0,07	0,23	0,18
beta-1	-0,07	0,15	0,07	0,23	0,10	0,16	0,10	0,35	0,35*	-0,02	0,19	0,07	0,09	0,02	0,28	-0,05	0,05
beta- 2	0,31*	0,14	-0,11	0,07	0,30*	0,22	0,22	0,28	0,35*	-0,06	0,19	-0,03	0,09	0,37*	0,16	-0,12	-0,01
gamma	-0,15	-0,23	-0,02	-0,10	0,06	-0,00	0,10	0,16	0,22	-0,08	-0,09	0,02	-0,03	0,09	-0,11	0,07	-0,04
APPT	-0,14	-0,19	-0,33*	-0,14	-0,02	-0,21	-0,15	0,02	0,13	0,07	-0,25	0,11	0,01	-0,06	-0,23	0,10	-0,12
Plasminogen	0,27*	0,31*	0,30*	0,35*	0,19	0,30*	0,19	0,28*	0,23	-0,06	0,31*	-0,08	0,00	0,05	1,00	-0,05	0,12
Platelet	-0,27*	-0,32*	-0,16	0,13	-0,35*	-0,31*	-0,31*	-0,13	-0,14	0,15	-0,29*	-0,21	-0,30*	-0,04	-0,05	1,00	-0,40
MPV	0,31*	0,31*	0,30*	0,01	0,28*	0,23	0,21	0,06	-0,04	0,03	0,24	-0,02	0,14	0,13	0,12	-0,40*	1,00

Annotation: the noted correlations are marked (*– p<0,05).

The correlation analysis noted the differences in the character and strength of correlation between metabolic and hemostatic parameters in the observed groups. In this way in the control inverse correlations were identified between HOMA-IR and HDL (r=-0,59, p<0,05), FPG et TC (r=-0,56, p<0,05). In addition there is no correlation between the parameters of metabolism and hemostasis in healthy patients, except for positive correlation between APTT and TC, LDL (r=0,55, r=0,76, respectively p<0,05).

In patients who have IFG, correlations lose their significant differences and are characterized by direct relationships between HOMA-IR and fasting plasma glucose (r=0,64, p<0,05), TG (r=0,82, p<0,05), LDL (r=0,81p<0,05), FPG and TG, LDL (r=0,71, r=0,70, respectively, p<0,05) and negative by relationships between FPG and ADP-induced platelet aggregation (r=-0,64, p<0,05), HbA_{1c} level positively correlates with fibrinogen and plasminogen (r=0,65, r=0,85, respectively, p<0,05).

In IGT subjects positive correlations are registered between HbA_{1c} and TG (r=0,52, p<0,05), APTT and TC, LDL (r=0,70, r=0,64, respectively, p<0,05) and negative correlations — between HbA_{1c} and MPV (r=-0,52, p<0,05), and also HOMA-IR and plasminogen (r=-0,67, p<0,05), number of platelets and their mean volume (r=-0,55, p<0,05).

ND T2DM is associated with a strong positive correlation between FPG and HbA1, TC (r=0,62, r=0,54, respectively, p<0,05), number of platelets and ADP, antithrombin III (r=0,57, r=0,69, p<0,05) and negative — between FPG and APTT (r=-0,56, p<0,05), TC and INR (International Normalized Ratio (r=-0,57, p<0,05), plasminogen and CT (r=-0,60, p<0,05).

Discussion

According to the results of our research, pre-diabetes and ND T2DM were diagnosed in the patients having excess body mass and WC. We did not find carbohydrate metabolism disorders among the participants of our research with excess BMI of 18,5-24,9 kg/m^2, women`s WC <88 cm, and men`s WC <102 cm. Hyperinsulinism, insulin resistance, increasing triglyceride concentration were observed in the patients with IFG, IGT and were maximally shown in patients with ND T2DM. TC level was significantly higher than standard indicators in IGT and ND T2DM, while elevated TC level in patients with IFG was not statistically significant.

Metabolic syndrome is characterized by dyslipidemia, high triglycerides in very low density lipoproteins and low HDL cholesterol. Hypertrygliceridemia in T2DM is associated with lower sensitivity of visceral fat tissue to the antilipolytic effect of insulin with boosted lypolises and the transport of free fatty acids into portal blood flow. In patients with hyperinsulinemia, these factors increase hepatic triglyceride synthesis and very low density lipoproteins synthesis of liver [10]. Under hyperglycemia the activity of endothelial lipoproteinlipasa decreases and that of hepatic lipoproteinlipasa increases, these processes being accompanied by suppressing triglycerides and by LDL catabolism by faster decomposing HDL cholesterol.

Therefore, changes of lipid profile are associated with insulin resistance, hyperinsulinemia and impaired carbohydrate exchange. We found close correlations between IMT, WC (reflecting the visceral fat mass) and HOMA-IR, FPG, TC, LDL, HDL, TG, corresponding to the current opinion about the role of metabolic syndrome in the development of T2DM and suggesting the presence of leading risk factors of atherosclerosis development at the earlier stages of overt T2DM [11,12].

Metabolic changes affect hemostasis. The absence of correlation between metabolic and hemostatic parameters in control and their presence in ICE patients show that biochemical changes play a key role in the initiating development hemostasis disorders.

According to "The IDF Consensus worldwide definition of the metabolic syndrome" (2006), the changes in fibrinolytic system (PAI etc) and coagulation (fibrinogen etc) are related to "Platinum standard" of the metabolic syndrome. In our research the tendency towards the increase of plasminogen level was found out in patients with IFG and IGT and was statistically significant in patients with ND T2DM. Strong correlations between HbA1c and plasminogen were observed in patients with IFG. The IGT stage demonstrates inverse mathematical, but direct functional correlation between insulin resistance and plasminogen. These direct correlations between plasminogen and concentration of FGP, TC, C-peptide, TG (Table 2) prove the multifactorial effect of the changed metabolic parameters on fibrinolysis. The obtained data can be explained by the results of the experimental studies, which showed that insulin and glucose participate in the regulation of plasminogen activator inhibitor-1 (PAI 1) gene expression as well as the effect of insulin on the synthesis of PAI-1 by a hepatocellular cell line [13,14]. According to Juhan-Vague and colleagues increased PAI-1 is a constituent of insulin resistance syndrome and adds thrombotic component into the traditional list of risk factors for atheromatosis. Triglycerides, LDL, and lower sensitivity of glycated plasminogen to pro-fibrinolytic enzymes with resistance to degradation are considered to be the other metabolic reasons changing fibrinolytic activity [15-16].

Hyperfibrinogenemia is considered to be an independent prognostic factor of atherosclerotic vascular disease [17]. Most studies on the hemostasis in patients with T2DM noted the mean increase of plasma fibrinogen concentration by 100 mg/dl.18 Although the elevated level of fibrinogen in all ICE-groups was not statistically significant, it showed strong correlations between HbA_{1c} and plasminogen in patients with IFG. Our data agree with the study results of Corrado and colleagues [18] who found a correlation between the duration of disease, concentration of glycated hemoglobin and fibrinogen increase. According to Dunn and colleagues [19], the formation of glycated fibrinogen leads to fibrin clots, which are resistant to plasmin. Therefore suppressing of fibrinolysis in T2DM, underlined by many authors, may be related to both the decrease of fibrinolytic potential and also to the changes in fibrinogen properties.

Platelets are rather vulnerable components of hemostasis in patients with ICE. 20 In addition, hyperinsulinemia, hyperglycemia and changes of lipid profile have a modulating effect on different levels of the platelet hemostasis.

The effect of chronic hyperglycemia on thrombocytopoesis brings about the appearance of large, young thrombocytic forms with high activity [21]. Increased glucose influx into megacariocytes significantly contributes to the increased thrombin-evoked depletion of cyclic nucleotides, and also increased activity of enzymes involved in glycolytic, acetyl-CoA synthesis, and fatty acid–synthesizing pathways [22]. Insulin also stimulates cell cycle and the processes of megakaryocytic cell-line differentiation [23]. The previously reported data about the influence of insulin and hyperglycemia on thrombocytopoiesis make possible to explain the tendency for the appearance in the blood flow of young, high-activity thrombocytic forms in patients with pre-diabetes as well as statistically significant increase of MPV in ND T2DM.

Chronic hyperglycemia leads to both the modulating effect on thrombocytopoiesis and the direct effect on platelets. Intracellular glucose concentration in a platelet is comparable with its extracellular concentration and associated with excessive formation of superoxide anion, protein kinase activity and decrease of NO [24]. The direct osmotic effect, i.e. exposition of platelets to hypertonic solution of glucose or mannitol solution during one hour is accompanied by activation of platelet glycoprotein IIb/IIIa and by release reaction of α-granules is considered to be another possible direct mechanism [25].

Low antiplatelet effect of insulin caused by its deficit or insulin resistance especially contributes to the development of platelet dysfunction in patients with T2DM. In vitro studies demonstrate that physiological concentrations of insulin have a direct inhibitory effect on platelet activity of healthy people. However, supraphysiological concentrations of the hormone and insulin resistance increase insulin-mediated platelet activity [26].

Platelet activity is also affected by lipid metabolizm disorders. The vast majority of experimental studies acknowledge the increase of platelet sensitivity to the aggregating agents in patients with atherogenic hyperlipidemia [27]. The previous reports show that cholesterol enrichment of membranes modifies platelet activity. Moreover they are associated with a 35-fold increase in sensitivity to epinephrine and 15-fold increase sensitivity to ADP and in higher sensitivity to collagen [28]. Acidity and microviscosity of platelet membrane are closely related to the concentration of plasma triglycerides. Dutu and colleagues [29] demonstrated the correlation between platelet adhesiveness and the level of free fatty acids in patients with hyperlipidemia. However, in the present

study we did not find the significant correlation between platelet activity and TC, TG concentration. Our findings are comparable with the findings of Jovan and colleagues [30] and can be explained by the changes in plasma fatty acid composition. Direct correlations between ADP-aggregation and concentration of beta2-globulines, identified in our study indirectly confirm the effect of changed lipid profile.

Hyperactive platelets initiate coagulation. A lot of data reported in literature confirm the activation of coagulation in patients with T2DM [31, 32]. Our study identified that changes in carbohydrate and lipid metabolism affect the coagulation system. According to the results of correlation analysis, the patients with IGT had close correlations between APPT and TC, and also between APPT and LDL. However, ND T2DM-patients had inverse mathematical but direct functional correlations between APPT and FPG, and between TC and INR. Nevertheless, biochemical analysis of the coagulogramms didn't identify any signs of coagulation. Shortened APPT in patients with ND T2DM and in patients with ICE in our study didn't reach statistical significance. The same results, showing the trend to activation of only some blood coagulation components in pre-diabetes, were reported by Maschrow and colleagues [33].

Thus, metabolic disorders that appear at the early stages of T2DM bring about changes in the hemostasis. Insulin resistance which is developed in people with visceral type of fat deposition in case of IGT and ITG is commonly accompanied by peculiar changes in carbohydrate metabolism and raised TC, TG concentrations. Metabolic disorders initiate non-significant changes in plasma and thrombocytic components of the hemostasis at the early stages of pre-diabetes. ND T2DM is associated with changes of lipid profile similar to pre-diabetes and 2-fold increase of insulin resistance in addition the increase of MPV and concentrations of plasminogen becomes statistically significant.

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