UDC 616.127-005.8: 615.355

EVALUATION AND ANALYSIS OF THE INVOLVEMENT OF A FIBRINOLYSIS INHIBITOR OF THE COAGULATION SYSTEM IN THE PROGRESSION OF CORONARY HEART DISEASE CHD

[1]Nurillaeva Nargiza, [2]Zokirova Mubarakkhon, [3]Khasanova Nargiza
[1,2,3] Tashkent Medical Academy

https://doi.org/10.5281/zenodo.7772057

Abstract. *Thrombin-activated Fibrinolysis inhibitor (TAFI), participates in the regulation of the balance between coagulation and fibrinolysis. High plasma levels of TAFI may therefore contribute to a hyperfibrinolytic condition and an increased risk of thrombotic disorders. Coronary stenosis is a consequence of the progression of atherosclerotic plaques, which is associated with a violation of fibrinolysis. Thrombin-activated fibrinolysis inhibitor (TAFI) and plasminogen activator 1 inhibitor (PAI-1) are fibrinolysis inhibitors whose levels depend on acquired conditions and polymorphisms. Therefore, our study is aimed at studying the association of TAFI gene polymorphism with the severity of coronary stenosis in patients with stable coronary artery disease (CAD).*

The methods that have recently appeared for determining alleles of polymorphic genetic markers using polymerase chain reaction will allow us to assess the genetic risk of developing cardiovascular pathology especially for the purpose of primary prevention of coronary heart disease, as well as to improve new approaches to individualization of therapy as a secondary prevention of the disease.

Keywords: *cardiovascular disease, ischemic heart disease, risk factors for the development of cardiovascular disease, myocardial infarction, t-PA - tissue plasminogen activator; TAFI, thrombin-activated fibrinolytic inhibitor; TAFIa is a thrombin-activated fibrinolytic inhibitor.*

Relevance. Cardiovascular diseases (CVD), in particular coronary heart disease (CHD), are still the leading causes of death and disability in many countries of the world [1,7]. Non-communicable diseases such as cancer, cardiovascular disease, diabetes and chronic respiratory disease are closely associated with risk factors such as tobacco and alcohol use, unhealthy diets and physical inactivity. Approximately 79% of all deaths in Uzbekistan are due to non-communicable diseases, and diseases of the cardiovascular system remain the main cause of premature death. [8,3]. The analysis shows that 53 percent of deaths among the population aged 30-70 years are associated with cardiovascular studieddiseases. The number of cardiovascular diseases in Uzbekistan has increased by 20% over 5 years. They account for more than 50% of deaths [9]. For the first time, the distribution of frequencies of alleles and genotypes of the thrombin-activated fibrinolysis inhibitor TAFI gene was studied, and the relationship between the prevailing risk factors for coronary artery disease and molecular genetic changes in blood coagulation factors was for the first time, the combination of the TAFI gene with traditional risk factors (RF) was evaluated, and its contribution to the total risk of CHD was revealed. It is known that disorders of the hemostasis system, leading to increased intravascular coagulation and thrombosis, are one of the most important links in pathogenesis CHD. In thrombosis, the balance

between hemcoagulation factors and the fibrinolysis system plays a leading role. Polymorphism of genes encoding factors of this system also plays a significant role in the processes of hemostasis. These genes can be considered as candidates for studying hereditary predisposition to CHD.

Identification of genetic factors and assessment of their contribution to the development of CVD are the main tasks of modern molecular cardiology. Polymorphisms in several hundred genes have been studied as genetic risk factors for atherosclerosis, arterial hypertension (AH), coronary heart disease (CHD), myocardial infarction (MI), stroke, thrombotic and other diseases.

The purpose of the study: To evaluate and analyze the association and role of thrombin-activated fibrinolysis inhibitor (TAFI) with risk factors and severity of CHD.

Material and methods. The object of the study were 87 persons who voluntarily signed up for inclusion in the study. The study was conducted by a prospective method in surgery of the Tashkent Medical Academy from 2020 to 2021. Statistical processing of income was obtained using Microsoft Excel spreadsheets and statistical software packages STATISTICA 6.0, SAS 6.3. An electronic database was opened using Excel Microsoft Office 2012. Statistical estimate of the value of income was obtained by the average estimate between the error on the t-Student's criterion.

Results and discussions. The study studied the associative relationship and role of thrombin-activated fibrinolysis inhibitor (TAFI) with risk factors and severity of coronary artery disease, since modern ideas about coagulation balance disorders are more informative in the development, course and prognosis of coronary heart disease. At the same time, the identification of genetic factors and the assessment of their contribution to the development of CVD are the main tasks of modern molecular cardiology. The main criteria for inclusion in the study was the presence of an established diagnosis in patients with coronary artery disease: Stable exertional angina (SEA) II-III FC. The functional class of SSN was established on the basis of the classification of angina pectoris of the Canadian Society of Cardiology (1976). The diagnosis of CHF was made on the basis of complaints (3 or more episodes of angina pectoris per week), clinical picture (attacks of anginal pain, lasting up to 15 minutes, stopped by taking nitroglycerin, occurring during physical activity when walking more than 500 m in FC II and within 100-200 m in FC III), history, physical examination, laboratory (lipid spectrum, coagulogram) and instrumental methods (VEM) in accordance with the recommendations of ESH / ESC (2019) [12, p. 425] and RCS/WHO (2017) [4], Patients with unstable angina in the second group were of comparable age and the diagnosis was made on the basis of the Braunwald classification. The study (2020-2021) included 87 individuals, of which 68 patients with coronary artery disease, both sexes, aged 48 to 76 years (average 61.9 ± 1.31) were included for further work. The group of healthy individuals consisted of 19 people aged 40 to 65 years (average 52.5±1.67 years) who did not suffer from CVD. The patients were divided into 2 groups: group 1 - 35 patients with IHD and stable exertional angina FC 1-4; Group 2 - 33 patients with coronary heart disease, unstable angina pectoris, with risk factors for the underlying disease [1]. All patients underwent a genetic blood test for TAFI gene polymorphism. All 68 patients received antiplatelet therapy, after which the frequency of adverse events (cardiovascular death, recurrent MI, stroke, bleeding) was assessed. The results of the study were described as M±SD (M is the arithmetic mean and SD is the standard deviation) or M±m (m is the standard error of the mean). For a statistical description of the relationship between different parameters, the Spearman rank correlation coefficient was calculated. Odds ratios (OR) corresponding to 95% confidence interval (CI) were calculated based on logistic regression

models. The level of statistical significance was considered to be p<0.05. Study of TAFI gene polymorphism, allele frequency and linkage disequilibrium between polymorphisms. For genotyping, out of 19 healthy and 68 patients with coronary artery disease (CVD and SP), 52 (60%) patients (out of 87 study respondents) with a pure Uzbek pedigree and who gave written informed consent were included. The clinical characteristics of 13 healthy individuals (68.4%) and 39 patients (57.4%) of IHD of Uzbek nationality were studied, taking into account significant risk factors. According to the data of clinical parameters of genotyped individuals, the association of non-modifiable and significant predominance of the most leading modifiable risk factors for coronary artery disease (CVD), such as TDS, was proved in 69% of patients with CVS and SP, as well as in 38.5% of healthy individuals. The presence of hereditary burden both in healthy and in patients with CVS and SP is pronounced, which amounted to 61.5% and 44%, respectively. Smoking is also expressed in both groups of respondents, i.e. 31 and 41%. Genomic DNA was isolated from peripheral blood leukocytes by the salting out method [4]. The sequenced region of the promoter was carried out according to the generally accepted method for determining human TAFI [2]. The division into 10 overlapping fragments approximately 300 bp in length was carried out.

Analysis of the TAFI gene polymorphism revealed significant heterogeneity in the frequencies of pathological and normal genotypes in patients with coronary artery disease and healthy individuals (Table 1; Fig. 1). Patients with Uzbek ancestry included with informed consent in the study suffered from the following leading risk factors: in the control group, this is hereditary burden -61.5%, the presence of TDS - 38.5%, and cigarette smoking with a high level of nicotine addiction - 31% . In the group of patients, CHF and NS turned out to be the leading risk factors for coronary artery disease: the presence of TDS - 69%, hereditary burden - 44%, and cigarette smoking with high nicotine dependence - in 41% of cases [10].

Table 1

Clinical characteristics of genotyped healthy persons and patients with CAD, taking into account significant risk factors

Indicators	**Control group (n=13)**		**Patients with CAD: CVS and SP (n=39)**		P
	абс	%	абс	%	
The presence of a hereditary burden	8	61,5	17	44	>0,05
smoking cigarettes	4	31	16	41	>0,05
The presence of a pronounced TDS	5	38,5	27	69	<0,01
Presence of obesity	1	7,7	11	28,2	<0,001
Age (average, years)	28,8±1,4		53,9±3,6		<0,001
Duration of CAD (in Wed per year)	0		4,81±0,7		<0,001
XC, mmol/l	156±9,8		201,7±14,3		<0,01

Further, the distribution of allele frequencies and their variations were studied. It turned out that the occurrence of pathological alleles was low. According to foreign scientists in their studies, the frequency of the minor allele varied from 0.24 to 0.49.

Table 2

The frequency of the main haplotypes of the TAFI gene in the entire sample and the 1st and 3 st tertiles of the distribution of TAFI Ag

	Haplotype						Frequency
	2599	**- 2345**	**- 438**	**aa147**	**+1542**	**+ 1583**	Everybody
H1	r	2G	A	Ala	r	T	0,26
H2	r	2G	r	Ala	C	T	0,16
H3	C	2G	r	Ala	C	T	0,19
H4	C	1G	r	Thr	C	A	0,21

The 6 remaining polymorphisms generated 4 main haplotypes, which is more than 80% of all haplotypes observed in the entire sample. None of the other observed haplotypes had frequencies above 5%. Within the 4 major haplotypes, the -2345 2G/1G, Ala147Thr, and T+1583A polymorphisms were in full association. All polymorphisms were strongly associated with TAFI Ag levels (P<10-4). In all cases, the model was consistent with the additive effect of the allele on the log-transformed variable. Geometric means and 95% confidence intervals. The percentage of variation explained by genotypes varied from 48% for the C + 1542G polymorphism to 20% for the -2345 2G / 1G polymorphism, which practically coincides with the data of foreign scientists [2].

Table 3

Plasma TAFI levels (geometric mean, 95% CI) according to C+1542G and Ala147Thr genotypes

Genotype Ala147Thr	**C + 1542G**		
	CC	**CG**	**GG**
Ала / Ала	78,3	58,6	35,2
	(71,4–85,8)	(54,2–63,3)	(30.1-41.1)
	n = 23	n = 31	п = 8
Ala / Thr	98,5	68,7	
	(91,1-106,5)	(62,8–75,3)	-
	n = 31	n = 23	
Thr / Thr	114,4	89,1	
	п = 1	п = 1	-

Two patients carrying a combination of + 1542CC and 147Thr/Thr had a higher level of TAFI. However, it should be emphasized again that the effect of a single Ala147Thr polymorphism is indistinguishable from the effect of a haplotype combining the Ala147Thr, T+1583A, and -2345 2G/1G polymorphisms due to the strong association between the 3 polymorphisms. In the model assuming codominant effect of polymorphisms on log (TAFI): C+1542G effect P <10 -4, Ala147Thr effect P <10 -4, interaction P = 0.77 [5]. In our study, the occurrence of C + 1542G and Ala147Thr polymorphisms of TAFI levels in plasma was as follows (Table 4).

Table 4

Plasma TAFI levels according to C+1542G and Ala147Thr genotypes in patients with CVI and SP

Genotype Ala147Thr	C + 1542G		
	CC	CG	GG
Ала / Ала	78,3	58,6	35,2
	(71,4–85,8)	(54,2–63,3)	(30.1-41.1)
	n = 2	n = 1	п = 3
Ala / Thr	98,5	68,7	
	(91,1-106,5)	(62,8–75,3)	-
	n = 1	n = 2	
Thr / Thr	114,4	89,1	
	п = 1	п = 1	-

Thus, due to the discovery of missing pathological alleles in the TAFI gene (only in 6 people), we had to cite the data of foreign scientists [5,3], where the occurrence of all studied alleles in them is low, which confirms the presence of a low risk of developing venous thrombosis in patients with coronary artery disease (CVD and NS). Probably, the obtained result of the study is associated with a small number of patients who gave informed consent to participate in the study. In this regard, a conclusion was made about the possible contribution to the development of coronary artery disease of the TAFI protein indicator as the main participant in the coagulation cascade [4]. So, in our study, the leading risk factors were identified and an assessment of the correlation interdependence of the studied indicators was presented (Fig.1.)

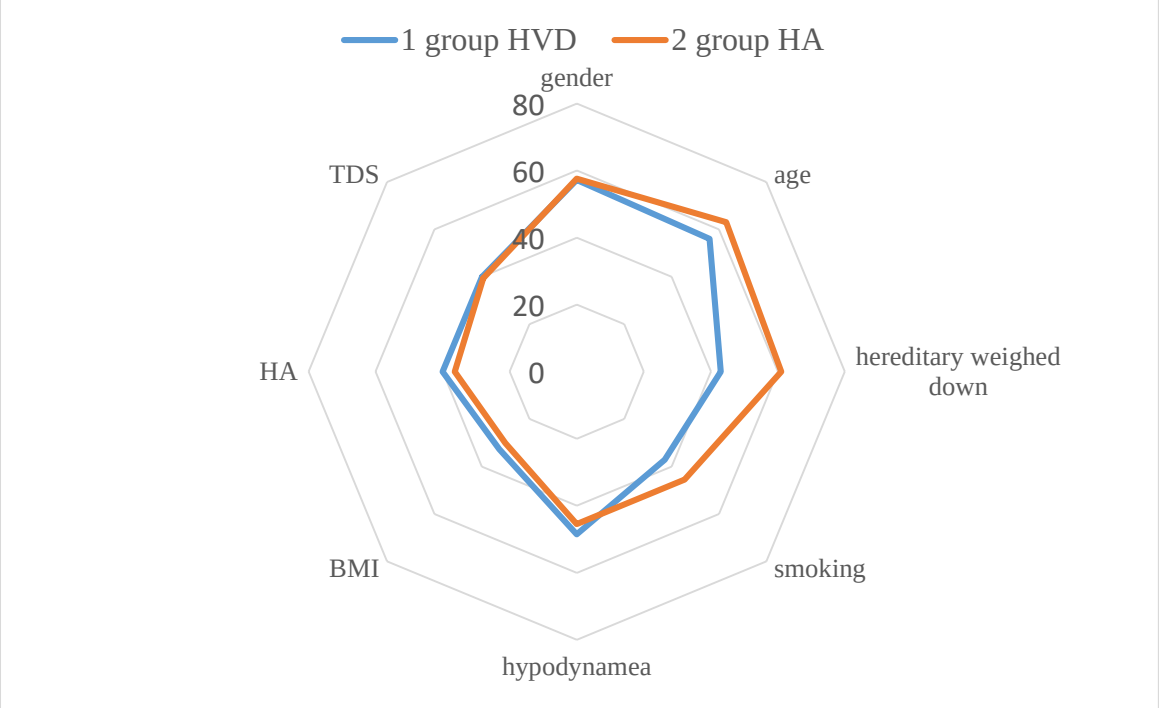

Figure 1. Leading risk factors and an assessment of the correlation of the studied indicators is presented

The study studied the state of coagulation hemostasis in patients with CHF and NS. In this regard, we have identified changes in coagulation hemostasis in patients of both groups and the control group. Since, in both groups, there were no distinguishable indicators for the components

of hemostasis, we decided to present the average values of changes in platelet hemostasis in patients with CHF and NS in both groups.

Table 5

Characteristics of platelet and coagulation hemostasis in study respondents

Indicators	Healthy, n=19	P
platelets ($10^{9/л}$)	187±15,2	
Platelet aggregation	6	
Start time VSK (min): the end:	3^{3}±0,1 4^{5}±0,1	
PTI (%)	70,8±3,1	
APTT **(сек.)	31,0±0,2	
Fibrinogen (мг/л)	2,9±0,2	
Hematocrit (%)	32,3±1,2	
Thrombomodulin (IU)	36,5 ±0,4	±2,3

According to some scientific studies, the degree of TAFI activation differs significantly between thrombin, plasmin and the thrombin/thrombomodulin complex, various experiments show that they all play a role in the physiological activation of TAFI. The results of the characteristics of platelet and coagulation hemostasis in the respondents of our study showed that the most significant significant difference was in terms of platelets by 28±...109/l, there was a significant decrease in APTT by 6.6 sec. Thrombomodulin, which activates TAFI activation, was significantly higher in patients with CHF and NS than in the healthy group by 23 IU. The presented study determined the correlation between risk factors for coronary artery disease and its clinical and laboratory parameters, where it turned out that there is a direct strong correlation between almost all indicators, except for weak links with HChS and partly TDS (Figure 2).

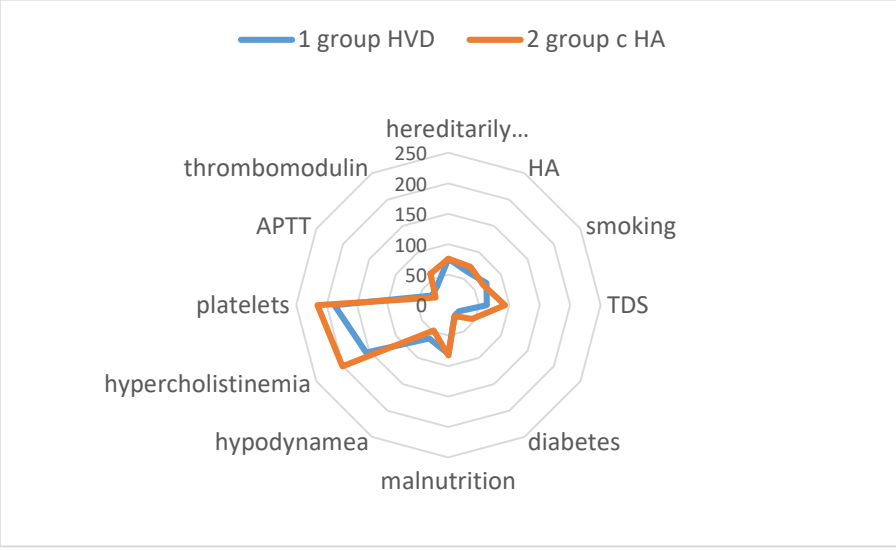

Figure 2. The presence of a correlation between risk factors for coronary artery disease and its clinical and laboratory parameters

The main problem in assessing the risk of developing CVD is the fragmentation and heterogeneity of markers used as risk factors. There is a large amount of literature data on individual genes involved in the pathogenesis of CHD. Phenotypic features that are taken into account by clinicians when predicting cardiovascular events are also described. However, a single approach that allows analyzing a relatively large number of polymorphic DNA markers in

combination with the most important phenotypic characteristics has not yet been developed, although this would significantly increase the effectiveness of assessing the individual risk of developing coronary artery disease [6]. Location in the same plane (according to Fig. 2), factors of coagulation hemostasis, as well as hereditary predisposition, hypertension and smoking indicate possible thrombotic events and the development of treatment tactics and preventive measures for patients with NS and CHF. According to the data of the basic therapy that our patients received, it turned out that in a larger percentage of cases, patients of group 1 received antiplatelet agents (91.4%), BABs (80%) and nitrates (43%). In the 2nd group of patients, patients with NS received the most antiplatelet agents (94%), BABs (91.0%) and nitrates (88%), as well as statins (91%).

Table 6

Information on basic therapy for patients of both groups

Drug group	1 group with SSN, n=35	2 group with NA, n=33
Nitrates	15 (43%)	29 (88%)
β-blockers	28 (80%)	30 (91%)
Ca antagonists	6 (17,1%)	8 (24,2%)
ACE inhibitor	7 (20%)	9 (27,3%)
ARA II	11 (31,4%)	12 (36,4%)
Antiplatelet agents	32 (91,4%)	31 (94%)

Quantitative characteristics of patients consuming drugs shows that patients in both groups take 2 to 4 drugs per day in accordance with clinical recommendations for the management of patients with coronary artery disease and hypertension, at individually indicated doses (Table 6). At the same time, the analysis of medication intake by patients of both groups showed that doctors comply with the standards of therapy, but the transition of patients from CHF to NA is possibly associated with the principles of taking medications (multicomponent therapy), adherence to therapy, the effect of risk factors on the clinical course of coronary artery disease, adherence to to invasive methods of treatment and many other factors (in particular, polymorphism of the genes of patients with CHF, NS) [8]. To conduct high-quality drug therapy, the so-called pharmacogenetic therapy, it is necessary to take into account genetic polymorphism, in particular, in our work, excessive intake of antiplatelet agents without taking into account the fibrinolytic activity of TAFI will lead to undesirable outcomes and variability of previously prescribed therapy, an increase in the incidence of complications from taking antiplatelet agents and a decrease in adherence to therapy of our patients. Patients [9].

Conclusions

Low polymorphisms of the TAFI gene do not affect the incidence of fatal and non-fatal complications (cardiovascular death, recurrent MI, stroke) in patients with MI. The absence of pathological alleles in the TAFI gene (only in 6 people), which coincides with the data on occurrence with the data of foreign scientists, where they also have a low occurrence of all alleles studied, which confirms the low risk of venous thrombosis in patients with coronary artery disease (CVD and NS). According to the data of the basic therapy that the patients received, it turned out that in a larger percentage of cases, patients of group 1 received antiplatelet agents (91.4%), BABs (80%) and nitrates (43%). In group 2, patients with NS received the most antiplatelet agents (94%), BABs (91%) and nitrates (88%), as well as statins (91%). The purpose and part of the completed tasks of the study will lay the foundation for proving the contribution of the TAFI gene to the course and outcome of patients with coronary artery diseases: with stable and unstable angina pectoris. Identification of genetic markers of risk of heart attack and stroke is key to both risk

prediction and potential intervention to prevent future cardiovascular events; The low occurrence of TAPI gene polymorphism allows doctors to conduct inexpensive research methods in the form of a quantitative characteristic of the TAPI protein.

REFERENCES

1. Townsend N. Cardiovascular disease in Europe: epidemiological update 2016.
2. Boffa M.B., Reid T.S., Joo E., Nesheim M.E., Koschinsky M.L. Characterization of the gene encoding human TAFI. Thrombin-activable fibrinolysis inhibitor; plasma procarboxypeptidase B) //Biochemistry.-38.-1999.- P. 6547- 6558.
3. Зокирова М.Б. Зависимость повышенной концентрации TAFI с сердечно-сосудистым риском. //XI Международный конгресс «Кардиология на перекрестке наук» 2021г. [Zokirova M.B. Zavisimost' povyshennoy kontsentratsii TAFI s serdechno-sosudistym riskom. XI Mezhdunarodnyy kongress «Kardiologiya na perekrestke nauk» 2021g].
4. Miller S.A., Dykes D.D., Polesky H.F. A simple salting out procedure for extracting DNA from human nucleated cells //Nucleic Acid Res.16.-1988.-1215].
5. M. Henry, H. Aubert, P.E. Morange, I. Nanni, Marie-Christine Alessi, L.Tiret, I.Juhan-Vague. Identification of polymer-phisms in the promoter and the 3′ region of the *TAFI* gene: evidence that plasma TAFI antigen levels are strongly genetically controlled //*Blood (*2001) 97 (7): 2053–2058. https://doi.org/10.1182/blood.V97.7.2053
6. Нуриллаева Н.М., Оценка взаимосвязанного влияния полиморфизма генов системы гемостаза и доминирующих факторов риска на раннюю манифестацию ишемической болезни сердца. //Евразийский Союз Ученых (ЕСУ) № 4 (13), 2015 | Медицинские науки. Стр 142-145 [Nurillayeva N.M., Otsenka vzaimosvyazannogo vliyaniya polimorfizma genov sistemy gemostaza i dominiruyushchikh faktorov riska na rannyuyu manifestatsiyu ishemicheskoy bolezni serdtsa. Yevraziyskiy Soyuz Uchenykh (YESU) № 4 (13), 2015 | Meditsinskiye nauki. Str 142-145]
7. Nick Townsend, Lauren Wilson, Prachi Bhatnagar, Kremlin Wickramasinghe , Mike Rayner , Melanie Nichols //https://www.ncbi.nlm.nih.gov/Cardiovascular disease in Europe: epidemiological update 2016. //www.ncbi.nlm.nih.gov/https://www.altmetric.com/details/10514271
8. https://news.un.org/ru/story/2021/01/1394212, WHO,2017
9. https://podrobno.uz/cat/obchestvo/neuteshitelnaya-statistika-chislo-serdechno-sosudistykh-zabolevaniy-v-uzbekistane-za-5-let-vyroslo-n.
10. Zokirova Mubarakkhon – To study the involvement of a fibrinolysis inhibitor of the coagulation system in the progression of coronary heart disease CHD //New Day in Medicine 12(50)2022 198-205 https://newdaymedicine.com/index.php/2022/12/26 Entered 10.01.2023