DIAGNOSTIC VALUE OF ENDOTHELIN 1 AND VASCULAR ENDOTHELIUM GROWTH FACTOR AND SIGNIFICANCE FOR DETERMINING THE THERAPEUTIC EFFECTIVENESS OF L-ARGININE ASPARATE IN CORONARY HEART DISEASE

[1]Alyavi A.L., [2]Tulyaganova D.K., [3]Toshev B.B.
State Institution "Republican Specialized Scientific and Practical Medical Center for Therapy and Medical Rehabilitation", Tashkent, Uzbekistan
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Abstract. *Coronary heart disease is the most common cause of death in the world. According to WHO, 740 million people die every year in the world, of which 13.2% die due to coronary heart disease. Therefore, the search for new markers that make it possible to predict coronary heart disease before an accident, as well as monitoring the therapeutic effectiveness of methods is one of the important and effective ways to reduce mortality. Through our study, we found that VEGF and ET-1 factor analysis is a very reliable candidate for predicting CAD and a very useful factor for evaluating the therapeutic effect of L-argiginine aspartate.*
Keywords: *VEGF; ET-1; Myocardial infarction; cardiac ischemia; L-arginine aspartate; EZVD.*

Introduction.

Vascular endothelial growth factor (VEGF) was discovered as a signaling molecule that increases vascular permeability by breaking intercellular contacts [1,3,5,8]. VEGF-A induces myocardial angiogenesis and increases vascular permeability and BM proliferation [2,6,7,12]. CMs are not only producers, but also targets for VEGF-A. A rat model has shown that VEGF-A inhibits apoptosis and activates the expression of genes involved in BM metabolism and contraction [9,10]. Under conditions of myocardial repair, VEGF-A promotes stem cell migration through the PI3K/Akt pathway [6,11].

In conditions of inflammation and neoplasia, isolated VEGF-A can be released by proteases, in particular metatalloproteinases, plasmin, urokinase-like plasminogen activator, elastase and tissue kallikrein. These proteases increase the activity of VEGF-A by influencing the clearance of the molecule, its activation and degradation, which activates angiogenesis, a key component of carcinogenesis, and can also suppress the VEGF angiogenic effect.

Materials and methods.

The study included 52 patients with coronary artery disease who were undergoing outpatient observation at the N.A. Semashko Ministry of Health of the Republic of Uzbekistan. The diagnosis was based on the clinical picture - clinical signs of angina pectoris of functional classes II-III, a history of myocardial infarction (MI) or electrocardiographic signs. Verification of the diagnosis was based on coronary angiography and coronary revascularization.

The average age of the patients was 55.94±1.29 years, height – 170.24±1.12 cm, weight – 77.72±1.79 kg. The study included 20 healthy volunteers without signs of damage to the cardiovascular system, comparable age and anthropometric characteristics, as a control group (CG).

In all patients, the presence of underlying diseases was recorded - arterial hypertension, type 2 diabetes mellitus, insulin resistance syndrome (according to the insulin resistance index in patients with glycemic levels within reference standards), hyperuricemia.

Laboratory research

VEGF concentrations were determined in serum obtained from peripheral venous blood. The concentration of VEGF was measured by enzyme-linked immunosorbent assay using the VEGF-ELISA-BEST reagent kit produced by Vector-Best CJSC (Russia) with a measurement range of 6.25-4000 pg/ml, the reference norm in blood serum is 6.25-600 pg /ml.

The concentration of ET-1 was determined in peripheral venous blood obtained by vacutainer from the cubital vein. The measurement was carried out by enzyme immunoassay; the range of values was 0-10 fmol/l.

Endothelial function. The assessment of endothelium-dependent vasodilation was based on the change in brachial artery diameter at 5 seconds after artery decompression during a 5-minute cuff test. The diameter of the artery was determined sonographically at 2 cm above the elbow. An ultrasound scanner equipped with a linear transducer with a frequency of 15 MHz was used. After measuring the diameter of the brachial artery and the maximum systolic blood flow velocity above the location site (on the shoulder), a tonometer cuff was applied and the pressure was injected 50 mmHg above systolic (PA 0). The compression was held for 5 minutes, after which the cuff was removed (sharp decompression) and at the 5th and 60th seconds the diameter of the brachial artery and the maximum blood flow velocity (PA 1) were re-measured. The dynamics of brachial artery diameter and flow velocity were recorded as a relative change expressed as a percentage.

After the initial examination, all patients included in the study were additionally included in the treatment regimen with L-arginine aspartate in a daily dose of 3 g. per day in 3 doses. The observation period was 3 months, after which a control examination of the condition of the myocardium and endothelium was carried out. Thus, the effectiveness of L-arginine aspartate in terms of endothelial and myocardial ischemic dysfunction in patients with coronary artery disease was studied, including depending on the concentration of VEGF and ET-1.

Results.

The study found that humoral markers of endothelial dysfunction were significantly increased in patients with coronary artery disease compared to a group of healthy volunteers (Table 1). Thus, the concentration of VEGF in patients with coronary artery disease was increased by 10.1 times ($p<0.001$), ET-1 – by 9.68 times ($p<0.001$).

Table 1

Comparative characteristics of the structural and functional state of the endothelium in patients with coronary heart disease and healthy individuals

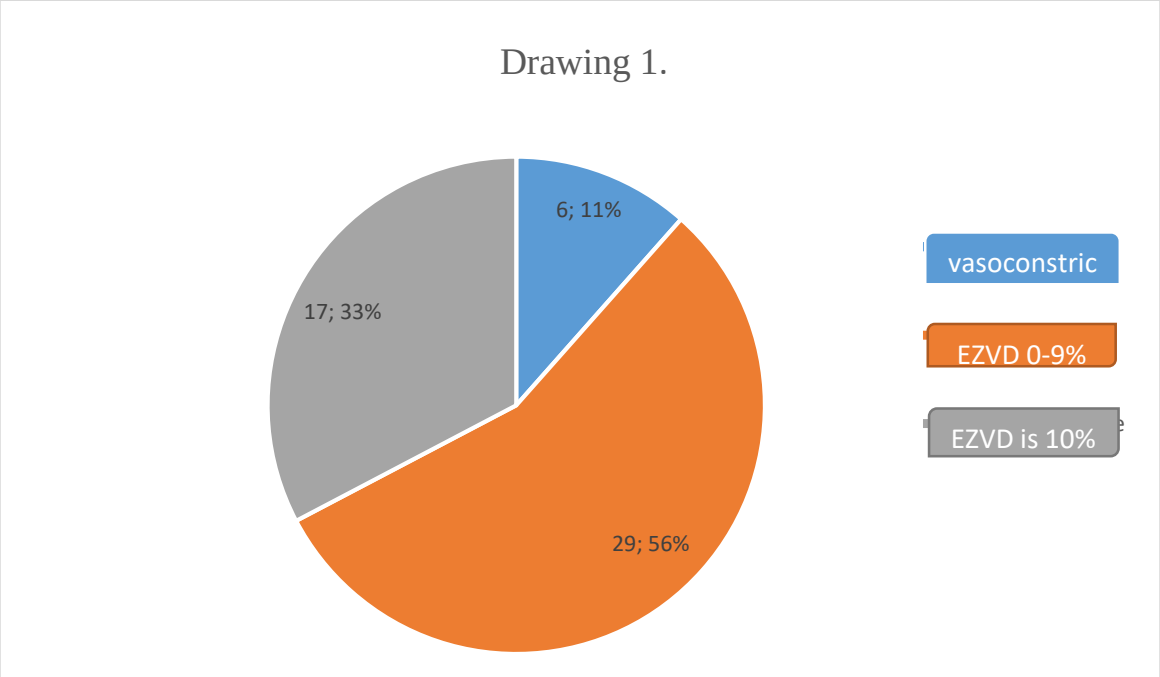
Sign	CG (n=20)	IHD (n=52)
VEGF, pg/ml	76,45±10,90	774,10±96,70[a]
ET-1, fmol/l	0,22±0,02	2,13±0,21[a]
PA 0, mm	4,19±0,15	4,17±0,10
PA 1, mm	4,35±0,15	4,25±0,10
EDVD, %	16,55±0,46	7,73±0,89[a]

Note: a - significance of the difference between groups – $p<0.05$.

In patients with IHD, despite the development of endothelial dysfunction, the diameter of the VA did not differ from the diameter of the vessel in the group of healthy individuals, both

initially and after 5-minute compression of the artery (results of VA in patients with IHD compared with the group of healthy volunteers) (Table 1).

In the group of patients with coronary artery disease, the majority of patients (55.5%) had insufficient EDVD (less than 10%), and in a smaller number of patients (33%), more than 10% of EDVD and 11% of patients had paradoxical vasoconstriction (Fig. 1).



1-drawing. Distribution of patients with coronary artery disease depending on the response of VA to the cuff compression test.

In addition, when the patients we studied were divided into groups depending on the results of EDVD, we found that patients with EDVD with 1-9% and patients with vasoconstriction showed that the markers VEGF, ET-1, PA and PA 1 were 3. 1 ($p<0.05$) and 8, 7 ($p<0.05$); 1.48 ($p<0.05$) and 2.78 ($p<0.05$); 1.044 ($p>0.05$) and 1.035 ($p>0.05$); 1.03 ($p>0.05$) and 1.005 ($p>0.05$) times higher compared to patients with a result of 10% or more EDVD (Table 2).

Table 2

Functional state of the endothelium and myocardium in patients with coronary artery disease depending on endothelial endothelium and myocardium

Sign	10% or more (n=29)	1-9% (n=17)	0% (n=6)
VEGF, pg/ml	268,36±44,40	848,66±76,13[a]	2336,50±178,61[ab]
ET-1, fmol/l	1,52±0,26	2,26±0,16	4,22±0,35[ab]
PA 0, mm	4,06±0,11	4,24±0,10	4,20±0,22
PA 1, mm	4,19±0,11	4,30±0,10	4,21±0,22

Note: a - reliability of results in relation to 1-9% EDVD; – $p<0.05$; b - reliability of the results relative to 0% EVD – $p<0.05$.

Correlation analysis showed that there is a significant negative relationship between the value of endothelial dysfunction and the concentration of endothelial dysfunction markers: strong with the concentration of VEGF and moderate with the concentration of ET-1. Also, correlation analysis showed the presence of significant negative relationships between the blood flow velocity in the VA at all stages of the test with humoral markers of endothelial dysfunction, more

pronounced with the concentration of VEGF and less pronounced with the concentration of ET-1 (Table 3).

Table 3.

Correlation analysis between VEGF and ET with other indicators

Sign	VEGF	ET-1
VEGF	-	0,68[a]
ET-1	0,68[a]	-
EDVD, %	-0,80[a]	-0,61[a]
Vmax 0	-0,53[a]	-0,29[a]
Vmax 1	-0,58[a]	-0,34[a]
Vmax 2	-0,57[a]	-0,32[a]

Note: a - correlation significance – p<0.05. Vmax 0, Vmax 1 and Vmax 2 are the maximum speed in the initial conditions, 5th second and 60th second of decompression, respectively.

Moreover, by assessing the predictive performance (AUC) of VEGF and ET-1, statistically significant indices such as sensitivity (SE) and specificity (SP) as independent markers were determined to obtain the diagnostic performance of these markers for coronary artery disease (Table 4).

Table 4.

Prognostic efficacy of VEGF and ET-1 against coronary heart disease

Factors	SE	SP	AUC
VEGF	1,0	0,9	0,97
ET-1	1,0	0,8	0,95

Table 5.

Comparative dynamics of myocardial and endothelial function indices in patients with coronary artery disease on the background of the inclusion of L-arginine aspartate.

Sign	(n=52)		
	Originally	3 months	Relative dynamics
VEGF, pg/ml	774,10±96,70	734,92±95,46	-8,98±1,27
ET-1, fmol/l	2,13±0,21	1,71±0,21[a]	-45,59±6,89
PA 0, mm	4,17±0,10	5,03±0,11[a]	21,51±1,70
PA 1, mm	4,25±0,10	5,13±0,11[a]	21,52±1,66
EDVD, %	7,73±0,89	9,78±0,93[a]	24,85±3,87

Note: a - significance of the difference with the original data – p<0.05.

As shown in Table 5, the diagnostic performance of VEGF and ET-1 was on an excellent basis. (0.97 and 0.95, respectively) [18].

Post-therapy results. The results of the analysis of patients after treatment with L-arginine aspartate showed a significant decrease in the concentration of VEGF and ET-1 by 5% (p>0.05) and 20% (p<0.05), and the relative dynamics of these markers was -8.98 and -45.59 respectively (Table 5).

The PA rate increased both before and after (20% (p<0.05) and 21% (p<0.05), respectively) of cuff compression, and the degree of EDVD also increased during observation with a statistically significant difference (p<0.05).

Discussion. Our study, as mentioned earlier, showed a significant increase in the levels of VEGF and ET-1 markers, as well as a statistically significant decrease in the percentage of compensatory vasodilation after a 5-minute cuff test, indicating a decrease in the physiological adaptation of blood flow in the perfused tissue in patients. In addition, we found that the dysfunction of compensatory endothelial adaptation was aggravated depending on the increase in VEGF and ET-1 levels (especially due to the latter) (Tables 1 and 2). Since with an increase in the level of ET-1, vasoconstriction becomes more intense, since in chronic heart failure the RAAS system is more intensely activated and ET-1 expression is induced. Meanwhile, VEGF may play a compensatory role to enhance angiogenesis under hypoxic conditions. In addition, the negative correlation between VEGF, ET-1 and EVD is also one of the results that supports this idea (Table 3).

Our study also supports the idea that VEGF and ET-1 are very reliable factors for predicting coronary heart disease, since our diagnostic performance was excellent for both factors (Table 4). And our result after treatment with L-arginine aspartate showed that the level of ET-1 decreased significantly and EDV increased statistically significantly, while we did not find statistically significant changes in VEGF. Since L-arginine is an aspartate, meta-analytic work has shown that L-arginine supplementation may have a blood pressure (BP)-lowering effect in various populations. and an increase in EDV, which leads to normalization of endothelial function. Once blood flow is normalized, the compensatory increased VEGF may become less intense, as we have seen. This may be caused by the production of nitric oxide. Nitric oxide (NO) is a potent antihypertensive agent. NO is formed when L-arginine is converted to citrulline by NO synthases (NOS), and L-arginine aspartate is a source of L-arginine [16]. NO antagonizes the effects of angiotensin II on vascular tone, cell growth, and renal sodium excretion, and also inhibits the synthesis of angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptors [17], which leads to decreased ET-1 production and increased vasodilation.

Conclusion. Through our study, we found that in people who had myocardial infarction, VEGF and ET-1 were significantly increased ($p<0.05$), while EDV was markedly decreased ($p<0.05$). In addition, the severity of EDVD dysfunction is also positively associated with the levels of VEGF and ET-1. Also, the diagnostic performance of VEGF and ET-1 was of excellent quality for coronary artery disease (AUC: 0.97 and 0.95, respectively). In addition, when re-examining patients after treatment with L-arginine aspartate and observation for 3 months, we found a statistically significant decrease in ET-1 factors ($p<0.05$) and an increase in endothelial adaptive function ($p<0.05$) (EDD) , PA 0 ($p<0.05$) and PA 1 ($p<0.05$), but not by changes in VEGF ($p>0.05$).

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