COMPARATIVE ANALYSIS OF SERUM GROWTH FACTOR LEVELS IN CHILDREN WITH DIFFERENT TYPES OF CONGENITAL HEART DEFECTS

Sadullaeva Iroda Kurbanovna

Head of the Department of Propaedeutics of

Childhood Diseases and Pediatric Neurology

Bukhara State Medical Institute

Relevance: Congenital heart defects (CHDs) are structural abnormalities of the heart that develop during fetal growth in the mother's womb. These defects may affect the structure of the heart, the valves, the heart walls, or the major vessels leading to and from the heart.
Children with CHDs may have an altered immune status, especially when the defect is associated with severe impairment of circulation and cardiac function.

Objective: To determine the characteristics of serum levels of growth factors (bFGF, VEGF-A, and TGF-β) in children with acyanotic and cyanotic congenital heart defects (CHDs).

Materials and Methods: The study included 52 children diagnosed with CHDs: 28 with acyanotic (white-type) and 24 with cyanotic (blue-type) defects. The control group consisted of 28 practically healthy children of similar age. Serum concentrations of basic fibroblast growth factor (bFGF), vascular endothelial growth factor-A (VEGF-A), and transforming growth factor-beta (TGF-β) were measured using a solid-phase enzyme-linked immunosorbent assay (ELISA) with test kits from JSC “Vector-Best” (Novosibirsk, Russia).

Results: Hypersecretion of bFGF, VEGF-A, and TGF-β was observed in patients from both CHD groups. Hypoxia, ischemia, compensatory angiogenesis, and depletion of cardiac tissue trigger activation of bFGF, VEGF-A, and TGF-β, which play a significant role in tissue regeneration and remodeling.

Keywords: congenital heart defects, children, growth factors, cyanosis, cytokines, serum, imbalance

Актуальность: Врожденные пороки сердца (ВПС) представляют собой структурные изменения в сердечном строении, которые образуются во время развития плода в утробе матери. Эти пороки могут затрагивать структуру сердца, клапаны, стенки сердца или крупные сосуды, ведущие к и из сердца.

У детей с ВПС может быть измененный иммунный статус, особенно если порок связан с тяжелым нарушением кровообращения и функционирования сердца.

ЦЕЛЬ:определение особенностей содержания факторов роста (bFGF, VEGF-A и TGF-β)в сыворотке крови у детей с ВПС белого и синего типов.

МАТЕРИАЛ И МЕТОДЫ: включены 52 ребенка с установленным диагнозом ВПС белого (28 пациентов) и синего (24 пациентов). Группу контроля составили 28 практически здоровых детей, аналогичного возраста. Концентрацию фактора роста фибробластов (bFGF), сосудисто-эндотелиального фактора роста-А (VEGF-A) и трансформирующего фактора роста-бета(TGF-β)в сыворотке крови определяли методом твердофазного иммуноферментного анализа с использованием тест-систем АО «Вектор-Бест» (Новосибирск, Россия).

РЕЗУЛЬТАТЫ: установлена гиперсекреция bFGF, VEGF-A и TGF-βу пациентов обеих групп с ВПС. Гипоксия, ишемия, компенсаторный ангиогенез, истощение сердечной ткани включают активацию bFGF, VEGF-A и TGF-β, которые играют важную роль в регенерации и ремоделировании тканей.

КЛЮЧЕВЫЕ СЛОВА: врожденные пороки сердца, дети, факторы роста, цианоз, цитокины, сыворотка, дисбаланс.

RELEVANCE: Congenital heart defects (CHDs) are structural abnormalities of the heart that develop during fetal growth in the mother's womb. These defects may affect the heart's structure, valves, walls, or the major vessels leading to and from the heart [1].

The most common defects include: ventricular septal defect (VSD) – 28.3%; atrial septal defect (ASD) – 10.3%; pulmonary artery stenosis – 9.8%; Tetralogy of Fallot (TOF) – 9.7%; aortic stenosis – 7.1%; aortic coarctation – 5.1%; transposition of the great arteries – 4.9%; as well as tricuspid valve hypoplasia, patent ductus arteriosus (PDA), and total anomalous pulmonary venous return [8]. More than 90 types of CHDs and numerous combinations of these defects have been reported.

At the A.N. Bakulev Institute of Cardiovascular Surgery, a classification system has been developed based on the distribution of CHDs, taking into account the anatomical features of the defect and hemodynamic disturbances. For practicing cardiologists, a simpler division of CHDs into three groups is more convenient:

Acyanotic CHDs with arteriovenous shunt: VSD, ASD, PDA; and atrioventricular canal (AVC).

Cyanotic CHDs with venous-to-arterial shunt: TGA, TOF, Fallot's triad, tricuspid valve atresia, etc.

CHDs without a shunt, but with obstruction to ventricular outflow: stenosis of the pulmonary artery and aorta.

This classification covers the nine most common CHDs [2,6].

Children with CHDs may have an altered immune status, especially when the defect is associated with severe impairment of circulation and cardiac function [3]. Based on the above, the objective of the present study is to determine the characteristics of serum growth factor levels in children with acyanotic and cyanotic CHDs.

Materials and Methods

This study included 52 children diagnosed with CHDs: 28 with acyanotic (white-type) and 24 with cyanotic (blue-type) defects. The control group consisted of 28 practically healthy boys and girls of similar age. Participants were aged from 1 to 12 years.

Immunological analyses were conducted in the Laboratory of Immunoregulation at the Institute of Immunology and Human Genomics, Academy of Sciences of the Republic of Uzbekistan.

Serum concentrations of basic fibroblast growth factor (bFGF), vascular endothelial growth factor-A (VEGF-A), and transforming growth factor-beta (TGF-β) in peripheral blood were determined using a solid-phase enzyme-linked immunosorbent assay (ELISA) with test kits from JSC "VECTOR-BEST" (Novosibirsk, Russia). Quantitative assessment of the results was performed using a calibration curve reflecting the relationship between optical density and the concentration of a standard antigen, allowing comparison with the tested samples.

Statistical analysis of the data was carried out using the software Statistica 6.0. Data were analyzed using standard methods, and results are presented as mean (M) ± standard error of the mean (m); median (Me) representing the central tendency; and upper and lower quartiles (Q1–Q3), reflecting the spread of values in 50% of respondents (Q1 — 25th percentile, Me — 50th percentile, Q3 — 75th percentile). The significance of differences between mean values (P) was assessed using Student's t-test.

Result sand Discussion:

It is well known that normal tissue function depends on the regular delivery of oxygen by the blood vessels. The process of neoangiogenesis is essential for long-term tissue adaptation under conditions of injury [12].

During tissue damage and inflammation, fibroblasts are activated by macrophages, secrete fibroblast growth factors (bFGF), and actively migrate to the site of injury. They attach to fibrillar structures via fibronectin while simultaneously synthesizing extracellular matrix components [14].

Fibroblasts secrete pro-angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and transforming growth factor-beta (TGF-β) [13].

Growth factors, typically classified as a subset of cytokines, are proteins that stimulate cell growth, differentiation, survival, inflammation, and tissue repair. They play a critical role in regulating various cellular processes and can be secreted by neighboring cells, distant tissues and glands, or even by tumor cells themselves. Normal cells require several growth factors to maintain proliferation and viability. Growth factors can exert their effects through endocrine, paracrine, or autocrine mechanisms [10].

Growth factors are involved not only in angiogenesis remodeling and immune responses but can also serve as biomarkers, as under certain pathological conditions they partially enter the bloodstream, which has diagnostic significance [10,12].

The results obtained are presented in Table 1 below.

Table 1.

Serum Levels of Growth Factors in Children with CHDs

Indicator	**M±m, pg/ml;**	**Me [Q1; Q3]**	**Min, pg/ml**	**Max, pg/ml**
Control group, n=28				
bFGF	6,21±0,28	6,65 [5,05; 7,31]	3,41	8,60
VEGF-A	34,01±1,42	33,90 [27,80; 39,11]	22,90	48,33
		47,55		

TGF-β	47,42±2,21	[36,52; 58,15]	28,73	66,90
B Acyanotic CHD (white type), n=28				
bFGF	29,28±2,02***	25,55 [20,77; 38,52]	14,30	48,51
VEGF-A	135,81±5,47***	144,75 [102,3; 159,07]	94,81	177,22
TGF-β	182,77±7,31***	191,15 [143,80; 214,62]	112,51	251,53
Cyanotic CHD (blue type)), n=24				
bFGF	34,51±1,68***	31,60 [29,35; 41,80]	20,10	49,71
VEGF-A	208,80±9,92***	200,55 [169,40; 246,05]	122,35	293,50
TGF-β	201,45±6,69***	200,05 [177,22; 226,85]	125,30	255,12

Note: ** — significant compared with the control group (* P<0.05, ** P<0.01, *** P<0.001). Me — median; Q1 (percentile) — 25th percentile; Q3 (percentile) — 75th percentile.*

Fibroblast growth factor (FGF) represents a family of cellular signaling proteins produced by various cell types [9]. One of its main functions is to stimulate the proliferation and migration of cells, including fibroblasts, endothelial cells, and cardiac myocytes.

As shown in Figure 1, analysis of serum bFGF concentrations revealed statistically significant results. The level of this growth factor was increased 4.7-fold, with a mean value of 29.28 ± 2.02 pg/mL and an individual range of 14.3 to 48.5 pg/mL, whereas in the group of healthy children, the mean level was 6.21 ± 0.28 pg/mL (P<0.001).

The elevated serum bFGF levels are likely due to tissue stress and damage. Increased bFGF synthesis is also probably involved in compensating for deficient blood supply in certain areas of the heart, which activates angiogenesis and releases bFGF to stimulate the growth of new blood vessels. This process, in turn, promotes remodeling of cardiac tissue, where thickening of the walls and myocardial hypertrophy may already be evident.

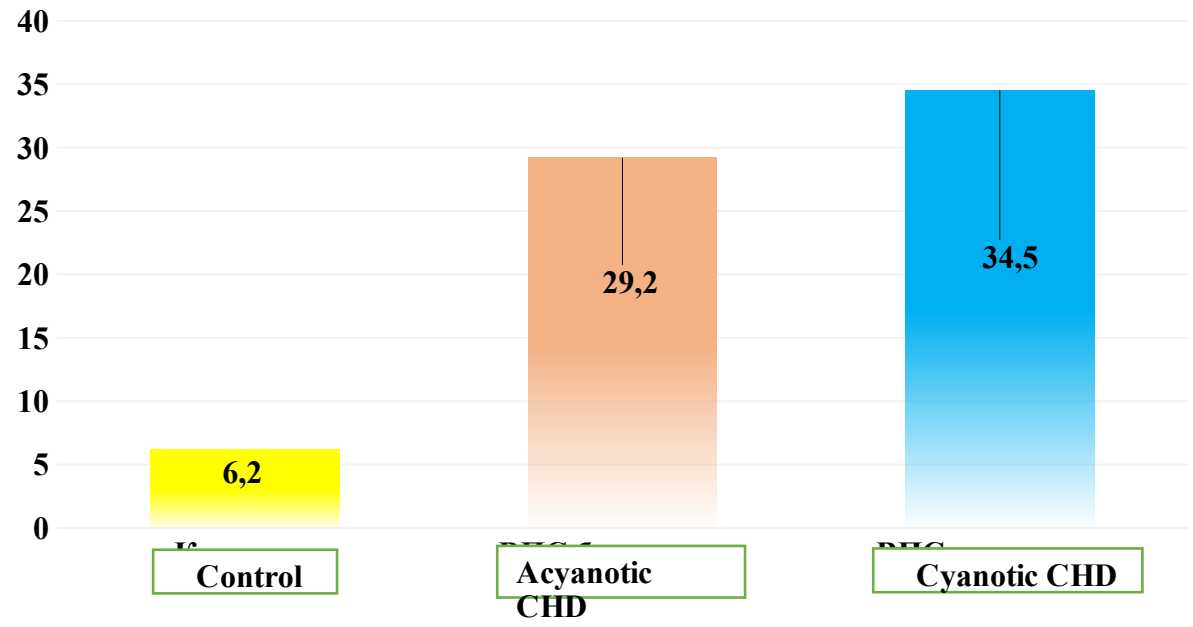

Figure 1. Serum bFGF Levels in the Examined Children Compared to the Control Group.

Note: * — *significant compared to the control group (* P<0.05, P<0.01, * P<0.001).*

As shown in Figure 1, analysis of serum bFGF concentrations revealed statistically significant results in the group of children with cyanotic CHD. The level of this growth factor was increased 5.6-fold, with a mean value of 34.51 ± 1.68 pg/mL and an individual range of 20.1 to 49.7 pg/mL, whereas in the group of healthy children, the mean level was 6.21 ± 0.28 pg/mL (P<0.001).

The elevated values likely indicate chronic hypoxia, which may have triggered enhanced expression. Under conditions of chronic hypoxia and tissue damage, fibrosis may also develop, affecting the structure and function of the heart, promoting angiogenesis, activating inflammatory processes, and remodeling tissues. These processes are part of the immune response and can influence the regulation of vessels and blood pressure in the heart and vasculature, which is also important in CHD.

Among the many pro-angiogenic factors involved in physiological and pathological angiogenesis, VEGF is the most important mediator of endothelial cell growth. VEGF is a family of structurally related proteins that, together with their receptors (VEGFR), play a key role in the development and regulation of blood and lymphatic vessels. The VEGF family includes several factors: VEGF-A, -B, -C, -D, -E, and placental growth factor (PlGF) [11]. VEGF-A, -B, and PlGF are the main regulators of blood vessel growth, while VEGF-C and -D are necessary for lymphatic vessel formation [7,11].

A key regulator of angiogenesis is VEGF-A. As a glycosylated mitogen, it specifically acts on endothelial cells and has pleiotropic effects, including increasing vascular wall permeability, inducing vasculogenesis, angiogenesis, and endothelial cell growth, and inhibiting their apoptosis [3]. Expression of this protein is stimulated both by numerous pro-angiogenic factors (EGF, PDGF, FGF, IL-1β) and by environmental conditions surrounding the cells (pressure, oxygen concentration, pH).

Assessment of serum VEGF-A concentrations in the group of children with acyanotic CHD revealed a significant increase. The synthesis of this growth factor increased 4-fold, with a mean value of 135.81 ± 5.47 pg/mL and a range of 94.8 to 177.2 pg/mL, compared to the normative values in healthy children, which averaged 34.01 ± 1.42 pg/mL (P<0.001) (Figure 2).

In our opinion, the elevated serum VEGF-A concentration in children with acyanotic CHD may be due to factors such as endothelial cell hyperactivity, angiogenesis, and vascular remodeling. These changes can lead to increased production and release of VEGF-A in an attempt to restore normal vascular structure and function.

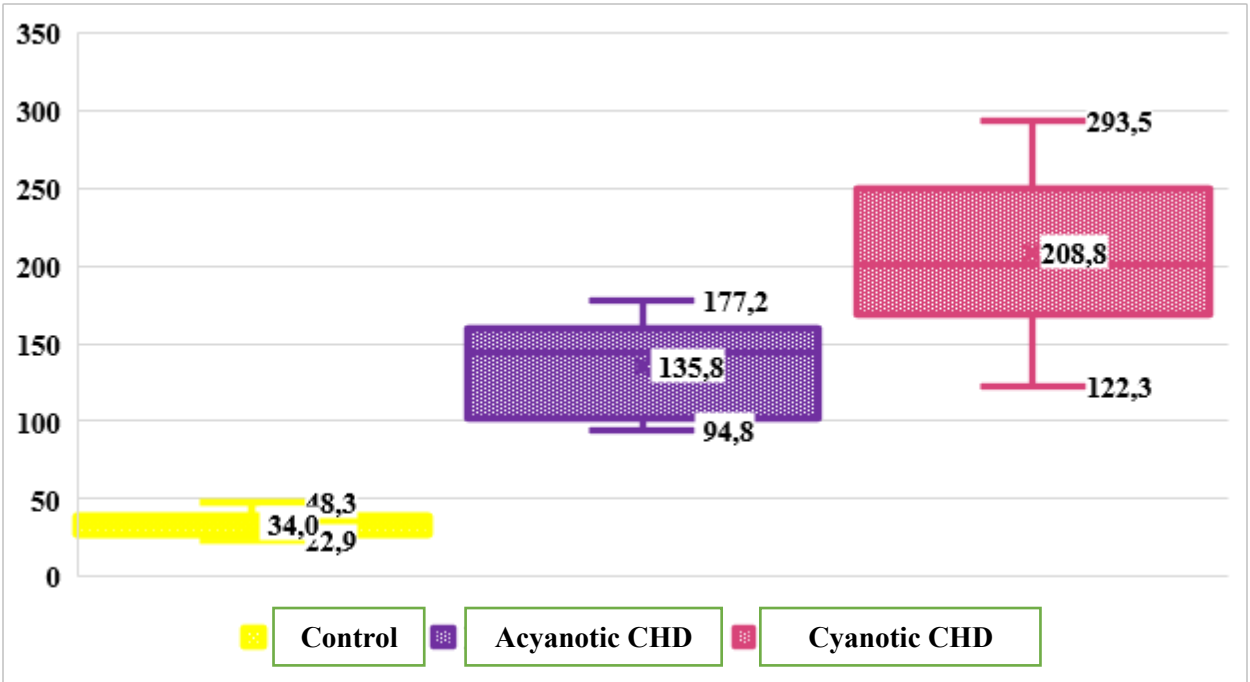

Figure 2. Serum VEGF-A Concentrations in the Examined Children Compared to the Control Group.

Note: * — significant compared to the control group (* P<0.05, P<0.01, * P<0.001).

According to the results shown in Figure 2, in the main group of children with cyanotic CHD, serum VEGF-A levels were significantly elevated. The synthesis of this growth factor increased 6.1-fold, with a mean value of 208.80 ±

9.92 pg/mL and a range of 122.3 to 293.5 pg/mL, compared to the normative values in healthy children, which averaged 34.01 ± 1.42 pg/mL (P<0.001).

These results suggest that in children with cyanosis, adaptive mechanisms are activated in response to processes such as chronic hypoxia, inflammatory reactions (e.g., in response to infections, tissue damage, or other pathological conditions), and tissue injury. These mechanisms aim to support tissue remodeling and angiogenesis for tissue repair and restoration, ensuring adequate blood supply to damaged tissues.

TGF-beta (transforming growth factor beta / TGF-β) is a type of cytokine that regulates proliferation, cell differentiation, and other functions in most cell types [15].

TGF-β is a factor synthesized in a wide variety of tissues. It acts synergistically with TGF-α, inducing phenotypic transformation, and can also function as a negative autocrine growth factor. TGF-β has diverse functions in the body. When acting on the immune system, its effects are predominantly inhibitory. Overall, TGF-β plays an important role in regulating various processes in the body, including immune response, tissue remodeling, wound healing, and anabolic processes [4,5].

Analysis of the results shown in Figure 3 revealed that TGF-β levels were elevated in the group of children with acyanotic CHD. The serum concentration of this growth factor in the main group of children was increased 3.9-fold, with a mean value of 182.7 ± 7.31 pg/mL and an individual range of 112.5 to 251.5 pg/mL, whereas in the group of practically healthy children, the mean level was 47.42 ± 2.21 pg/mL (P<0.001).

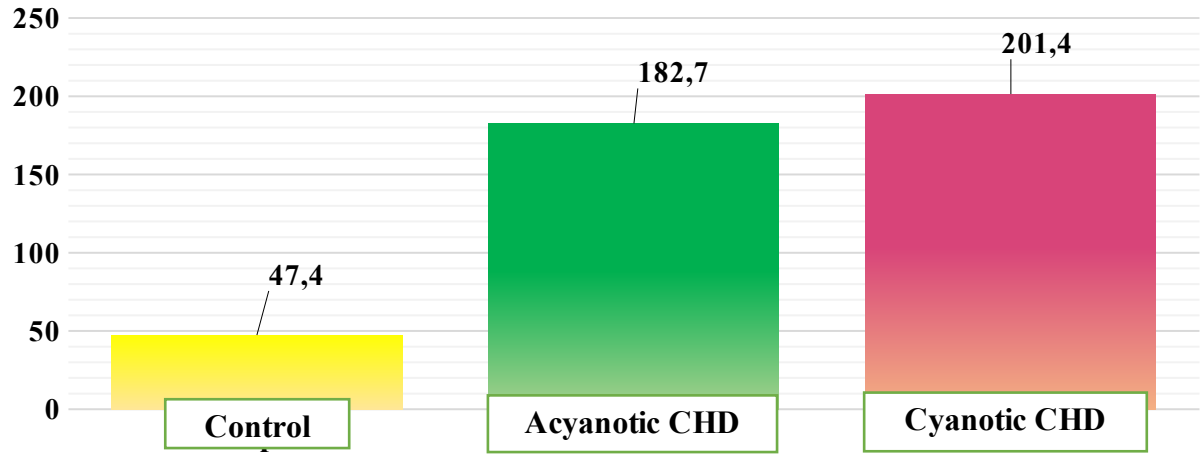

Figure 3. Serum TGF-β Levels in the Examined Children Compared to the Control Group.

Note: * — significant compared to the control group (* P<0.05, P<0.01, * P<0.001).

The results likely indicate that elevated serum TGF-β levels in children with acyanotic CHD may be associated with cardiac workload, inflammation, and tissue remodeling, as well as with the role of TGF-β in tissue development and differentiation. However, it should be noted that each case may have its specific characteristics, and further studies may be necessary to more precisely determine the cause of increased TGF-β levels.

Assessment of the results shown in Figure 3 revealed statistically significant TGF-β levels in the group of children with cyanotic CHD. The serum concentration of this growth factor in the main group was increased 4.2-fold, with a mean value of 201.45 ± 6.69 pg/mL and an individual range of 125.3 to 255.1 pg/mL, whereas in the group of practically healthy children, the mean level was 47.42 ± 2.21 pg/mL ($P<0.001$).

Based on the obtained results, we hypothesize that excessive TGF-β in the blood can have both protective and destructive effects on the body, depending on the context and duration of its action. The marked increase in serum TGF-β levels in children with cyanotic CHD may be due to factors such as hypoxia and ischemia in response to tissue damage and the body's attempt to adapt to the altered environment; inflammation and tissue remodeling; and mechanical factors, since congenital heart defects can cause increased cardiac and vascular load, which may activate TGF-β production in response to mechanical stress.

Thus, immunological studies revealed that hypoxia and ischemia in CHD, both with and without cyanosis, can stimulate the increased production of various cytokines and growth factors in response to tissue damage and stress. In turn, CHD and hypoxia can trigger remodeling of cardiac and vascular tissues. This process involves the activation of bFGF, VEGF-A, and TGF-β, which play important roles in tissue regeneration and remodeling.

CONCLUSION

The identified immunological changes trigger cascade reactions in the body aimed at adapting to oxygen and blood supply deficiencies. Activation of cells and tissues to cope with damage and stress leads to increased production of various biologically active substances. Further studies and clinical observations are necessary to more precisely understand the role of these mediators in the pathology of different types of CHD

LITERATURE

1. Белозеров, Ю.М. Детская кардиология /Ю.М. Белозеров. – М., 2004. – С. 9-221.
2. Бокерия, Л.А. Сердечно-сосудистая хирургия2001. Болезни и врожденные аномалии системы кровообращения /Л.А. Бокерия, Р.Г. Гудкова. – М., 2002. – 348 с.
3. Закирова Н.Э., Закирова А.Н. Роль иммуновоспалительных реакций и дисфункции эндотелия в ремоделировании миокарда и прогрессировании ИБС. Рациональная Фармакотерапия в Кардиологии. 2014;10(5):488-94]. DOI:10.20996/1819- 6446-2014-10-5-488-494.
4. Корженевская К. В., Гавришева Н. А., Панов А. В. и др. Трансформирующий фактор роста-β1 при различном клиническом течении ишемической болезни сердца после операции коронарного шунтирования. Медицинская Иммунология. 2010;12(6):521-8
5. Моисеева О.М., Шляхто Е.В. Трансформирующий фактор роста и маркеры активации лейкоцитов при гипертонической болезни. Артериальная Гипертензия. 2003;9(1):14-6]. DOI:10.18705/1607-419X-2003-9-1-14-16.
6. Подзолков В.П., Шведунова В.Н. Врожденные пороки сердца. РМЖ. 2001; 10: 430
7. Роль VEGF в развитии неопластического ангиогенеза / В. П. Чехонин [и др.] // Вестн. РАМН. — 2012. — № 2. — С. 23–34
8. Шарыкин, А.С. Перинатальная кардиология: руков. для педиатров, акушеров, неонатологов /А.С. Шарыкин. – М., 2007. – 264 с
9. Ardizzone A., Scuderi S.A., Giuffrida D., Colarossi C., Puglisi C., Campolo M., Cuzzocrea S., Esposito E., Paterniti I. Role of Fibroblast Growth Factors Receptors (FGFRs) in Brain Tumors, Focus on Astrocytoma and Glioblastoma. Cancers. 2020; 12:3825. doi: 10.3390/cancers12123825. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
10. Arita S., Kikkawa F., Kajiyama H., Shibata K., Kawai M., Mizuno K., Nagasaka T., Ino K., Nomura S. Prognostic importance of vascular endothelial growth factor and its receptors in the uterine sarcoma. Int. J. Gynecol. Cancer, 2005, Vol. 15, no. 2, pp. 329-336
11. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress / N. Ferrara // Endocr. Rev. — 2004. — Vol. 25. — P. 581–611
12. Flavell SJ, Hou TZ, Lax S, Filer AD, Salmon M, Buckley CD. Fibroblasts as novel therapeutic targets in chronic inflammation. British Journal of Pharmacology. 2008;153(S1): S241–S246.DOI: http://dx.doi.org/10.1038/sj.bjp.0707487

13. Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, Bucala R, et al. Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. FASEB journal. 2001 Oct;15(12):2215–2224. DOI: http://dx.doi.org/10.1096/fj.01-0049com

14. Keeley EC, Mehrad B, Strieter RM. Fibrocytes: Bringing new insights into mechanisms of inflammation and fibrosis. The international journal of biochemistry & cell biology. 2010 Apr;42(4): 535–542. DOI: http://dx.doi.org/10.1016/j.biocel.2009.10.014

15. Verrecchia F., Mauviel A. Transfoming growth factor-beta and fibrosis. World J Gastroenterol. 2007;47(8):13-18. DOI:10.3748/wjg. v13.i22.3056.