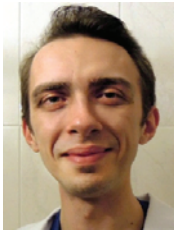
The results of diagnostic and treatment of patients with diabetic retinopathy and age-related macular degeneration at a diabetes type 2.





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SUMMARY

Purpose. Explore the changing clinical and functional and morphological changes of the retina against application of angiogenesis inhibitor in patients with diabetes type 2 with comorbidity fundus: diabetic retinopathy (DR) and age-related macular degeneration (AMD).

Methods. In the main study group included 22 patients (22 eyes) with type 2 diabetes with combined fundus pathology DR and AMD. All patients before and after intravitreal injection of an angiogenesis inhibitor ranibizumab (Lucentis, Novartis) was assessed visual acuity, macular thickness and macular morphology based on the results of OCT, the retinal sensitivity according to the data of microperimetry (MAIA). The control group study included 30 people (15 healthy and 15 with type 2 diabetes without DR).

Results. When comparing the main group with the control group was revealed that visual acuity in the main group (0,27±0,05) was significantly lower than in the control group (0,8±0,01, p <0.05); retinal thickness was significantly higher in the control group, and the retinal sensitivity was significantly lower. On the background of intravitreal injection of ranibizumab all patients with DR and AMD had significantly increase in visual acuity on average by 37% (from 0,27±0,05 before treatment to 0,37±0,05 after treatment), a significantly reduction of macular thickness in 9 out of 9 areas, including the fovea centralis, an average of 32.6% and increase retinal sensitivity by 24% (from 11,75±1,68 (dB) to 14,58±1,68 (dB), (p <0.05). The correlations were found between visual acuity and retinal thickness, as well as between visual acuity and retinal sensitivity of the macula, before treatment r = −0,26, p <0.01 and r = 0,7 p <0.01, respectively, after treatment with r = −0,14, p <0.01 and r = 0,64, p <0.01, respectively.

Conclusions. Intravitreal injection of angiogenesis inhibitor ranibizumab to patients with comorbidity fundus DR and AMD on a background of type 2 diabetes pathogenesis is justified and leads to a significant improvement in clinical and functional and morphological parameters of the retina.

Keywords: diabetic retinopathy, age-related macular degeneration, ranibizumab.

Financial Disclosure: No athor has a financial or property interest in any material or method mentioned.
There is no conflict of interests

Ophthalmology in Russia. — 2014. — Vol. 11,No 3. — P. 20–26

There are 45 million blind in the world for 2013 year according to WHO. Some of the leading causes of blindness are age-related macular degeneration (AMD) and diabetic retinopathy (DR) [1].

347 million people registered with diabetes mellitus (DM) in 2013 the world [2]. There are 2.8 million people with type 2 diabetes among the adult population on 2008 in Russia, according to the Federal Center of the State Register of diabetes (GRSD). DR is a microvascular complication of diabetes, the prevalence of diabetic retinopathy in diabetic patients according to some sources is more than 80% [3]. The average age of patients with type 2 diabetes in Russia is 60,5±0,62 years [4], this age group is also at risk for AMD.

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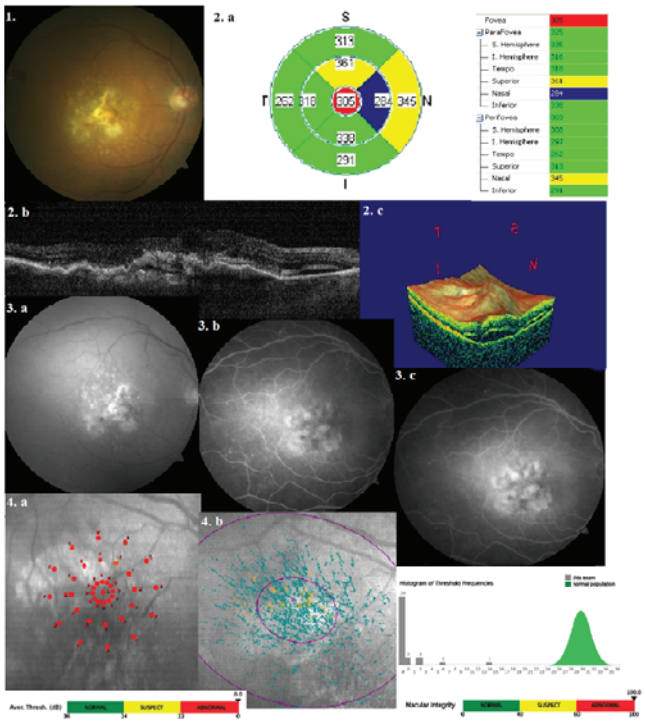

Fig. 1 Data before treatment: 1 — fundus image (Nidek-NM100); 2 — OCT (RTVue-100): a — macular thickness; b — line scan; c — 3 D macula; 3 (a, b, c) — FAG; 4 — mikroperimetriya (MAIA): a — map of macular sensitivity; b — fixation stability.

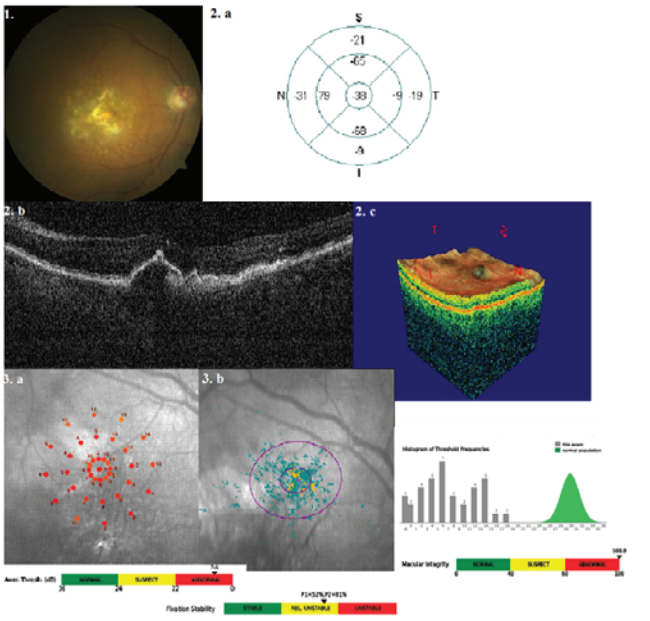

Fig. 2 Data after injection of ranibizumab (Lucentis) on 7 day: 1 — fundus image (Nidek-NM100); 2 — OCT (RTVue-100): a — changes of the macular thickness; b — line scan; c — 3 D macula; 3 — mikroperimetriya (MAIA): a — map of the macular sensitivity; b — fixation stability.

AMD is the leading cause of visual impairment in older individuals. More than 60 million people suffer from this disease in the world [5, 6, 7]. The incidence of AMD is more than 15 cases per 1000 population in Russia. [8]

The recent studies have shown that changes caused by DR and AMD can develop at the same time and the presence of DR is associated with a significant risk of «dry» (nonexudative) and «wet» (exudative) form of AMD [9].

WHO has identified DR and AMD as the priority diseases for the prevention of blindness and visual impairment in the developed countries [10].

Microvascular complications, associated with DR and diabetic macular edema (DME), are caused by chronic hyperglycemia, which causes damage and dysfunction of the endothelial cells of capillaries in the retina, as well as other common metabolic disorders, such as diabetic dyslipidemia, hypertension, and vascular inflammation [11, 12]. Over time, constantly growing microvascular damage leads to a cascade of pathological processes, expressed in the appearance of nonperfusion areas and the development ischemia of inner retina, increasing the activity of vascular endothelial growth factor (VEGF), the appearance of the macular edema and the retinal neovascularization. These processes can cause loss of vision and eventually to blindness [12]. Microvascular injury are also the cause of fluid leakage from the retinal capillaries and its excessive extracellular accumulation, that is leads to the development DME [10]. While the molecular pathogenesis of DME is not completely elucidated, it is believed that VEGF plays an important role in it [10].

The AMD pathogenesis combines several mechanisms, some of which have not been completely elucidated. Oxidative stress is an important factor contributing to the development of AMD. Formation of free radicals is due to increased synthesis of lipofuscin [13], increased levels of the enzyme myeloperoxidase [14], that leads to damage retinal pigment epithelium (RPE) and the disorder of their functions and, in turn, causes a disturbance of the nutrients transport from the choriocapillaris to outer retinal layers and, consequently, their hypoxia, which stimulates the release of VEGF and development of choroidal neovascular membrane (CNV) [14].

It is believed that changes in the gene factor H also increase the risk of developing AMD [15]. Factor H is one of the main inhibitory factor for the complement system and gene mutations, it damage can lead to sustained activation of the complement system, which causes atrophy of the RPE and photoreceptors, as well as changes in Bruch's membrane, leading to the development of CNV [15].

Development of CNV is presumably result of an increase of angiogenic stimuli, wich overcome antiangiogenic compensatory mechanisms in the eye. This imbalance may result from tissue hypoxia, inflammation, or combinations of both. VEGF has been detected in vitreous of patients with AMD in the presence of CNV to high concentrations, while the content of the pigment epithelium-derived factor (PEDF) with antiangiogenic properties were lower than normal [16].

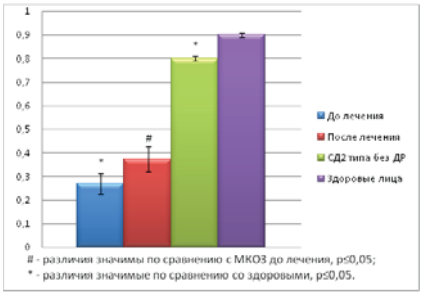

Fig. 3. The visual acuity dynamics before and after injection of ranibizumab (Lucentis) (M±m, p)

Table 1. Changes of the visual activity, the critical flicker frequency, the intraocular pressure and the macular thickness in 9 areas before and after injection of ranibizumab (Lucentis).

	Контрольная группа		Основная группа	
Показатели	Здоровые (M±m)	СД 2 типа без ДР(M±m, p)	До лечения(M±m, p)	После лечения(M±m, p)
МКОЗ	0,9±0,01	0,8±0,01, *p<0,05	0,27±0,05, #p<0,05	0,37±0,05, $p<0,01
КЧСМ	39,5±0,3	39,1±0,7, *p>0,05	36,9±0,3, #p>0,05	37,1±0,3, $p>0,05
ВГД	18,1±02	18,2±0,4, *p>0,05	18,3±0,3, #p>0,05	18,4±0,4, $p>0,05
Толщина сетчатки по данным ОКТ (мкм)				
Центр f. c.	250,43±2,51	247,81±4,12, *p>0,05	399,6±23,5, #p<0,05	301,3±16,4, $p<0,01
Parafovea (3mm)	260,68±3,36	283,19±5,09 *p<0,05	376,5±21,6, #p<0,05	313,1±11,9, $p<0,01
	272,00±2,92	282,53±10,1, *p<0,05	379,4±22,6, #p<0,05	321,2±12,7, $p<0,01
	270,06±2,95	298,84±2,38, *p<0,05	371,5±16,3, #p<0,05	321,0±12,9, $p<0,01
	274,50±2,30	289,22±3,32, *p<0,05	360,3±16,1, #p<0,05	308,0±9,8, $p<0,01
Perifovea (5mm)	280,9±1,92	256,84±4,73, *p<0,05	317,2±15,6, #p<0,05	288,0±9,8, $p<0,01
	287,9±1,37	270,25±5,16, *p<0,05	333,9±19,5, #p<0,05	293,5±11,3, $p<0,05
	286,00±1,17	275,69±3,37, *p>0,05	311,0±12,8, #p<0,05	298,0±11,8, $p<0,05
	284,87±1,68	247,00±3,35, *p<0,05	294,9±13,0, #p<0,05	277,7±9,3, $p<0,05

VEGF — is a powerful endothelial specific mitogen, it is a homodimer with a molecular weighing approximately 45 kDa [10]. VEGF is the mediator of many important physiological processes, including relating to the development and maintenance of vascularization [17], regulation of blood coagulation and vascular tone through the production of nitric oxide and prostacyclin I2 [17].

Human VEGF family includes five related glycoproteins: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor [17, 18, 19, 20]. VEGF-A, is mainly responsible for the development of blood vessels and vascular permeability in the adult [19, 20]. Alternative splicing and/or proteolytic cleavage of the eighth exon of VEGF-A gene gives four major isoforms VEGF-A: VEGF121, VEGF165 (most common), VEGF189, and VEGF206 [10, 17, 20]. All members of VEGF family transmit a signal via three transmembrane tyrosine kinases receptor (VEGFRs): VEGFR-1, VEGFR-2 and VEGFR-3 [17, 19, 20]. Most mitogenic and angiogenic responses to VEGF-A mediated through VEGFR-2, which expressed on vascular endothelium [10].

Ranibizumab is an optimized Fab-fragment of anti-VEGF-A antibody bevacizumab.. It binds to and blocks all isoforms of VEGF-A [21]. Ranibizumab approved for intraocular use in the treatment of AMD and macular edema secondary to retinal vein occlusion by FDA (Food and Drug Administration) [22].

Two of the largest studies, conducted in patients with AMD-MARINA (Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular Age-Related Macular Degeneration) and ANCHOR (Anti-VEGF Antibody for the Treatment of Predominantly Classic Choroidal Neovascularization in Age-Related Macular Degeneration) — proved the efficacy of ranibizumab to prevent loss of vision in patients with CNV [23, 24].

Several prospective clinical trials in patients with DME, including, READ-2, RESOLVE, RESTORE, DRCR.net Protocol I, and RISE/RIDE proved that the intravitreal injection of ranibizumab reduces macular edema and sustainably improves vision acute in these patients [25, 26, 27, 28, 29, 30, 31].

The purpose of the present work was a comparative study of clinical and functional parameters and morphological changes in the fundus of patients with combined fundus pathology — diabetic retinopathy and age-related macular degeneration with type 2 diabetes before and after intravitreal injection of an inhibitor angiogenesis — ranibizumab (Lucentis).

MATERIALS AND METHODS

22 patients (22 eyes) with AMD and DR in type 2 diabetes were included in the main study group. The control group consisted of 15 healthy patients (30 eyes) and 15 patients with type 2 diabetes without DR (30 eyes).

Ophthalmological examinations were conducted at the Department of Ophthalmology GBOU DPO RMAPE (Russian Medical Academy of Postgraduate Education) Russian Ministry of Health on the basis of out-patient department GBUZ Municipal Clinical Hospital S. P. Botkin branch #1.

The main study group included 17 women (75%) and 5 men (25%), the average age was — 70,7±1,3 years. Disease duration of type 2 diabetes up to 5 years was detected in 3 (5%) patients, 6-10 years — in 4 (20%), 11-15 years — in 7 (35%), more than 15 years — 6 (30%). Average fasting glucose was 6,9±0,2 (mmol/L), and glycated hemoglobin (HbA1s) — 7,13±0,21 (%).

All patients had DR and AMD, DR stage was evaluated by classifying E. Kohner and M. Porta, AMD — by

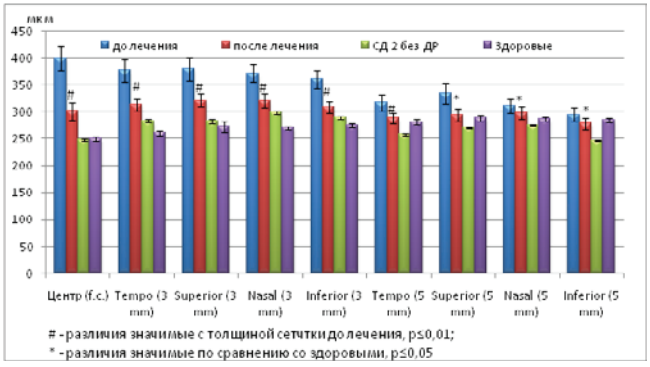

Fig. 4. The macular thickness (RTVue-100) before and after injection of ranibizumab (Lucentis), mkm (M±m, p).

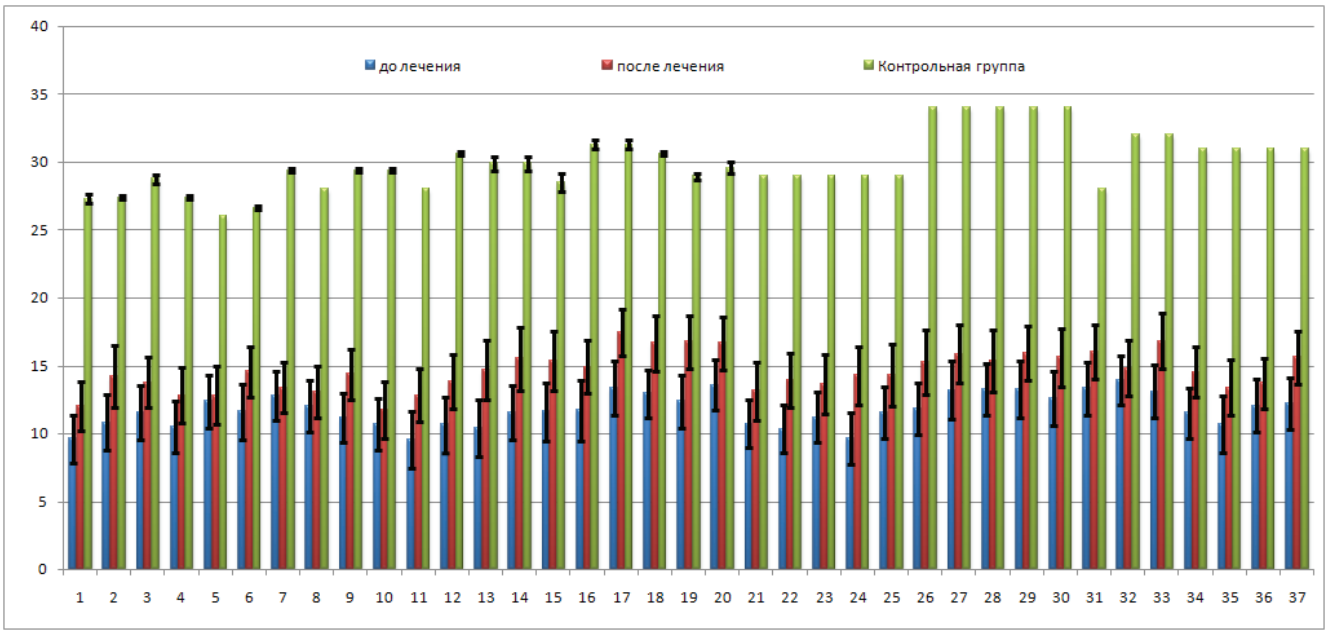

Fig. 5 The macular sensitivity (MAIA) before and after injection of ranibizumab (Lucentis), dB (M±m, p).

AREDS. DR I (nonproliferative DR) with clinically significant DME (diabetic macular edema) and AMD (AREDS 1, 2, 3) — identified in 8 (36%) eyes, DR I (nonproliferative DR) and AMD (AREDS 4) — 14 (64%) eyes, 5 of them was diagnosed clinically significant DME.

In addition to standard ophthalmologic examination (visometry by the usual method with optimal correction of ametropia to maximize visual acuity; perimetry by quantitative perimetry in eight meridians on the projection sferoperimetre; intraocular pressure by the standard method using applanation Maklakov's tonometer (load weight 10 g); the critical fusion frequency flicker (CFFF) — with an instrument «Flash-test») before and after treatment (day 7), all patients were also conducted fundus photoregistration using fundus Nidek-NM1000 (Nidek, Japan), optical coherence tomography (OCT) scanner using RTVue-100 (OptoVue, USA), mikroperimetry using mikroperimeter MAIA (CenterVue Spa, Italy).

Ophthalmoscopy: the optic nerve head was a pink color on 24 eyes (100%); vascular bundle was located in the center of the optic nerve head on 24 eyes (100%). Arteriovenous ratio was on average 0,5±0,04. Microaneurysms along the vascular arcades identified in 19 eyes (86%). Hard exudates was marked in the macular and paramacular areas on 20 eyes (90%), with characteristic localization in the form of a ring around the macula was observed on 3 eyes (13.6%), dry druze — in 8 (36%) eyes, choroidal neovascular membrane — in 8 (36%) eyes, retinal pigment epithelium (RPE) detachment — in 2 (9%) eyes, DME — in 13 (59%) eyes. Hemorrhages of varying severity (from the micro to like a flame) were noted in 20 eyes (90%).

All patients were injected inhibitor of angiogenesis — ranibizumab (Lucentis, Novartis Pharma, registration number: LSR-004567) on the basis of the ophthalmology department GBUZ Municipal Clinical Hospital #67 L. A. Vorohobov. Indications for the injection were clinically significant DME and/or exudative form of AMD (CNV, RPE detachment — AREDS 4).

Patient J., 81 years old, suffers type 2 diabetes for 30 years, stage of compensation. Fasting glucose — 7.0 mmol/l, glycated hemoglobin (HbA1c) — 7,5. Visual acuity before treatment was 0.1; biomicroscopic anterior segment of right eye was normal by age; ophthalmoscopy of right eye fundus — the optic nerve head was pink color, with accurate boundaries; arteries were sclerotic changed, veins were few full-blooded; macular area had extensive gray structure, displacement pigment. single microaneurysms, hemorrhages, soft exudates; paramacular and along the vessels were many microaneurysms and hemorrhage of various size. According to the results of OCT retinal thickness in the fovea centralis was 305 microns, with the violation of the RPE integrity, CNV and DME; according to fluorescein angiography (FAG) there are signs of microaneurysms along the vessels in the macular area; land of the capillary occlusion, signs of the colloid drusen, RPE hyperplasia, occult CNV, macular edema; according to the results of retinal sensitivity was sharply reduced and fixation was instable (Fig. 1). After injection of Lucentis on 7 day visual acuity increased up to 0.3, the retinal thickness decreased on 68 microns in the fovea according by OCT; retinal sensitivity increased and fixation became more stable according by microperimetry (Fig. 2).

Statistical analysis was performed using Microsoft Office Excel 2007. To calculate the reliability of differences used Student's t-test and U-test Mann-Whitney. The correlation coefficient was assessed by Pearson (r). Differences

were considered statistically significant when p <0,05.

RESULTS AND DISCUSSION

As seen from Table 1, the maximum corrected visual acuity (MCVA) in the study group before treatment was on average 0,27±0,05 and was significantly lower than MCVA 0,8±0,01 in patients with type 2 diabetes without DR (p <0.05) and 0,9±0,01 in healthy people (p <0.01). After injection of Lucentis on 7 day MCVA increased in all patients of the main group on average by 37% (from 0,27±0,05 before treatment to 0,37±0,05 after treatment), which was statistically significant (p <0,, 05) (Table 1, Fig. 3). Data of IOP (mm Hg. tbsp.) and CFFF (Hz) in the main and control groups did not differ and have not changed before and after therapy.

Study of retinal thickness in 9 areas in the control group showed significant differences in retinal thickness in patients with type 2 diabetes without DR and healthy individuals (p <0.05) in all areas except the fovea. In the main group before treatment retinal thickness was significantly higher than in diabetic patients without DR and healthy individuals (p <0.05). Comparative analysis of the main group conducted after treatment with Lucentis on 7 day showed, that the thickness of the retina according to OCT decreased significantly in 9 of 9 areas including a fovea centralis, where the thickness of the retina before treatment was 399,6±235 (microns), and after treatment — 301,3±16,4 (microns) (p <0.01), i.e. decreased by an average of 32.6% (Table 1, Figure 4).

Analysis of retinal sensitivity in macula showed significant decrease in the main group compared with the control (diabetes without DR and healthy people) in all 37 points (p <0.05). In the main group after treatment on 7 day showed a significant increase average retinal sensitivity on 24% (from 11,75±1,68 (dB) to 14,58±1,68 (dB) (p <0.05) (Figure. 5).

Correlation analysis conducted between MCVA and average thickness of the retina in the main group showed the presence of reliable negative weak relationship (r = –0,26, p <0.01) before treatment, as well as reliable negative weak relationship (r = –0,14, p <0.01) after therapy by Lucentis (Fig. 6). Between MCVA and retinal sensitivity of the macula revealed the presence of a reliable positive strong relationship (r = 0,7, p <0.01) before treatment and reliable positive medium strength relationship after treatment (r = 0,64, p <0.01) (Figure 7).

Results of screening patients with DR conducted to detect changes related to AMD showed that «dry» form of ARMD has been detected in 35% of examined (in the control group, 30%), and the «wet» form in 9% (in the control group, 6%) [9].

In the materials studied by us, we found no data for management and treatment of patients with this combined pathology. To date, a globally recognized standard treatment for patients with clinically significant DME (defined by Early Treatment of Diabetic Retinopathy Study (ETDRS) clini-

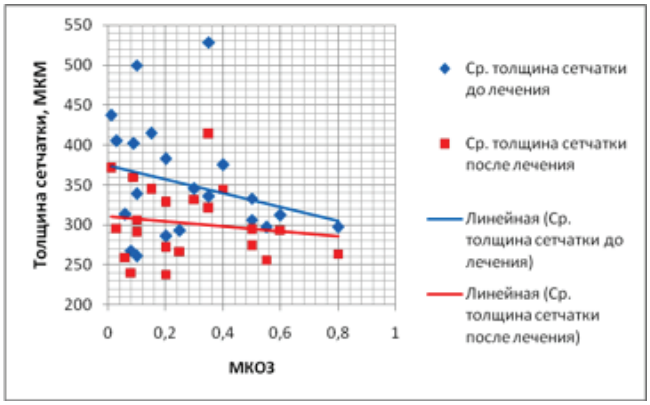

Fig. 6 Correlations between the visual activity and the macular thickness before and after injection of ranibizumab (Lucentis).

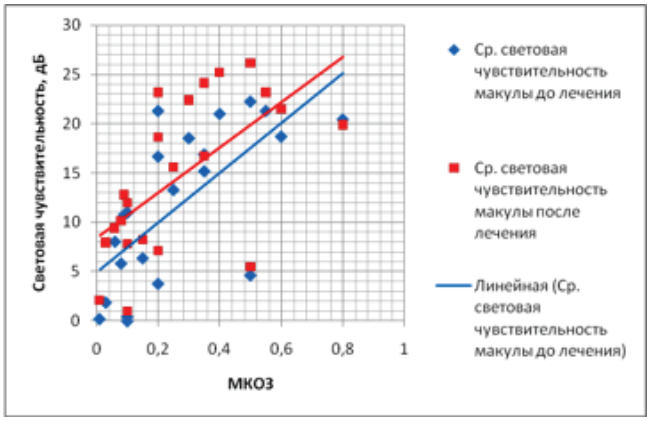

Fig. 7 Correlations between the visual activity and the macular sensetivity before and after injection of ranibizumab (Lucentis).

cally significant edema is determined by the presence of one of the three criteria: retinal thickening within 500 μm (1/3 of the optic disk) of the anatomical center of the macula; the formation of «hard» exudates in the macula, or within 500 μm from its center, in combination with macular edema; at least 1 disc area of retinal thickening from 500 to 1500 μm of the anatomical center of the macula) and «wet «form of AMD (corresponds to AREDS4) is intravitreal injection of anti-VEFG drugs [23, 24, 25, 26, 27, 28, 29, 30, 31].

Based on the foregoing, our study included patients with clinically significant DME and/or «wet» form of AMD (CNV, RPE detachment).

Our results showed a marked decrease of visual acuity in the main group, which is associated with the presence of a pathological process directly in the macular area. Increasing the thickness of the retina was mainly due to patients with clinically significant DME (average thickness of the retina before treatment was 399,6±23,5 μm).

Correlation analysis performed between visual acuity and retinal thickness before treatment showed the presence of reliable negative weak relationship (r = –0,26, p <0.01). This can be explained by very low visual acuity of patients with the predominance «wet» form of AMD (CNV) corresponded the smaller increasing retinal thickness, compared

with DME. A more weak correlation (r = –0,14, p <0.01) between these parameters after treatment explained by the fact that visual acuity of patients with the «wet» form of AMD (CNV) improved less than visual acuity of patients with DME and «dry «AMD (AREDS 2, 3).

The retinal sensitivity in the control group in all 37 points was higher than 25 dB, and in the main group before treatment was 11,75±1,68 (dB), which is more than two times lower than normal and points to the functional disorders macular area. After treatment, the sensitivity increased on average by 24%, indicating that the recovery of the morphology of the macula according to OCT leads to an increase in its objective functional abilities on the results of microperimetry.

Our study showed the need for deep (ophthalmic and biochemical) study of patients with combined pathology fundus DR and AMD, followed by analysis and interpretation of data, which can result in improved diagnosis, develop tactics and treatment of these patients. In this connection in subsequent publications planned presentation of the survey of patients with combined pathology at different stages of DR and AMD.

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CONCLUSION

Visual acute of patients with type 2 diabetes with DR and AMD was significantly lower, and the thickness of the retina was higher than in healthy individuals and patients with type 2 diabetes without DR (p <0.05).

Retinal sensitivity of macula according to microperimetry was significantly decreased (p <0.05) at patients with combined fundus pathology (DR and AMD) compared with the control group (patients with type 2 diabetes without DR and healthy individuals).

After injection of Lutsntis in the main group visual acute statistically significant increase (p <0.05), retinal thickness decrease in in all areas, including the fovea (p <0.01) and the average retinal sensitivity in the macula significant increase.

This research has proved that intravitreal injection of angiogenesis inhibitor is pathogenesis reasonable and effective treatment for patients with combined fundus pathology (DR and AMD).

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