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RECONSTRUCTIVE OPERATIONS IN AORTIC ROOF ANEURYMS WITH AORTAL INSUFFICIENCY

Abstract. Reconstructive surgery of the aortic root is of great interest in the field of cardiac surgery. Over the past three decades, a number of techniques, differing in technical performance and the anatomical area of the correction, have been proposed for the correction of aortic root aneurysm with aortic valve insufficiency. The purpose of these procedures is to maintain the functioning of the cusps and stabilize other components of the aortic root. Reliable and long-lasting effect of such interventions is particularly important due to the lack of necessity of taking anticoagulants. The choice of the method of surgical correction still remains controversial and in each case the surgeon is the one to make the decision. This review describes the methods of the aorta root reconstruction with a systematic approach of choosing a surgical correction method, accompanied by illustrations of the operations. The description of the procedures, followed by illustrations, facilitates the selection of the surgical method in each individual case.

Keywords: aortic root aneurysm, aortic insufficiency, reconstruction of aortic root.

Introduction. Most of the authors believed that the aortic aneurism is the local or widespread growth of it's diameter for 1.5 times (Svensson L.G., 1997, Покровский А.В. 1979, Белов Ю.В. 2011). In most cases aortic root aneurism accompanies aortic valve's inefficiency. The most frequent mechanisms of aortic regurgitation with aortic aneurism are annule aortal ectasia, expantion of sinotubular junction and sinus of Valsalva's aneurism [84].

Over the past two decades, a number of methods were suggested for correction of aortic insufficiency, differing technical implementation and the anatomical area of aortic root. The purpose of these technics is saving functional leaflets by valve repair, replacement or stabilization of aortic root's other components [1]. Reliable and long-lasting effect of these interventions particularly beneficial, in the absence of the necessity of receiving anticoagulants.

The purpose of this article is to review development of operation methods, definition of indication to the use of different methods, based on the restoration of the functional anatomy of the complex – "aortic root".

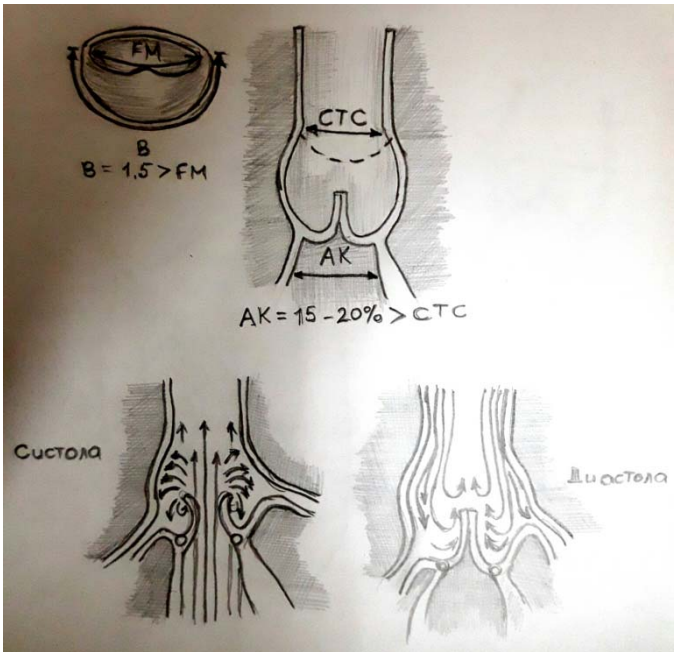
Functional anatomy of the complex – "aortic root" and aortic valve. In 1994, Kunzelman and co-authors published an important work describing the anatomy of aortic root and equilibration with aortic valve [2].

Finding of article was that the diameter of aortic root at the level of the middle Sinus was considered as 100%, and the diameter of theat level of the sinus pecten made up 81% of diameter and size of normal aortic root bases left 97% of the first indicator

In other words, diameter of cinotubular ridge is approximately 85% of the aortic ring's diameter at the base of the root.

This quantified analysis of the aortic root's anatomy certifies da Vinci's theory about on Vortex flows created by cinotubular ridge (figure 1). Fluid's vortex flows arising between the edges of the folds of the valve leaflets and aortic wall create two effects:

1. In opening phase these flows avert contact valve leaflets with the wall of the aorta.
2. In closing phase flows initiate closing of leaflets



Cosing of aortic valve and flow division

AA = 15 – 20 % > STJ

Figure 1 – Statistic and functional anatomy of aortic root.Sinotubular Junction's diameter (STJ) is less than aortic root's bottom (aortic rings – AA) for approximately 15%

The second effect described by authors, probably even more complicated than suggested,- the flow of the fluid causes the closing of valve on the sistoly's last moment and vortex fows protect leaflets from flexure and encourage their smooth and synchronized closure.

Dynamic anatomy of the aortic root. Dynamic Anatomy of the aortic root has been described for an understanding the mechanism of reducing stress on the leaf and thus avoiding aging and possible structural valve dysfunction [3-10]. A group of scientists from Stanford used radioactive marker on a model of sheep's aortic root, noting a number of complicated asymmetric deformations during the cardial cycle, involving junctional zones (alleged aortic rings) and sinotubular junction as well as protraction, compression, expansion and aortic root scretching [3]. Lansac and co-authors' Four-dimensional study of aortic root confirm's that aortic root expansion begins at its base in isovolemic reduction phase and hence extends to the comissure valve and ultimately-sinotubular junction [4]. Maximum expansion of the aortic root get at the first third of systole, approaching the cylindrical form, then to mid-diastolic pressure goes reduction in volume, and the root became conoid. Aortic root enlargement for 39% and 63% in the komissural area, gives improvement of the blood outflow to systole, as well as makes the work of sinistral

stomach more effective.Lansac and Dagum's research explains the importance of interpetaloid triangles described in the works of Anderson and co-authors , which clearly define aortic ring as a valid subaortic structure consisting of 3 basic interpetaloid triangles, similar to the rack attachment line cusps of the aortic valve [3, 4, 9, 10]. Aortic root base tends to grow in accordance with kinetics ventricle.

Aortic root expands upward through disruption of m interpetaloid triangles, however, sinuses and sinotubular ridge maximum expandes at the end of miocardia.

In addition, this aortic root's exact dynamic enlargement cycle, should be a specific chronology, also affecting to the position and degree of disjunction of the bottom on which attached leaflets of valves, as was first described Thubricar [10] (figure 1). All valve-preserving techniques, to varying degrees, change this dynamic geometry of the aortic root.

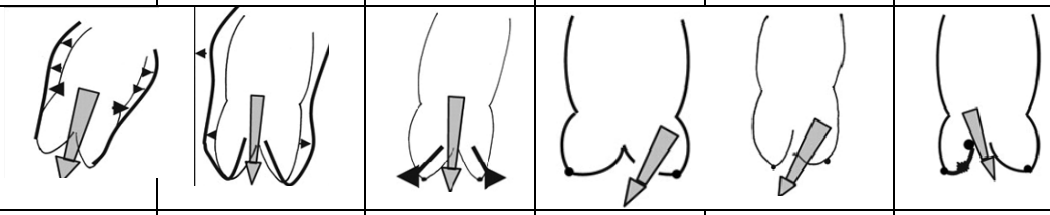
Diseases at which valve-sparing surgery are possible. Basically, all these reconstructive surgery are only applicable in cases where the aortic insufficiency is caused by the following lesions (table):

Aortic root enlargement repeated to the ascending aorta aneurism:

1. Mechanism of aortic insufficiency connected with the expansion of the sinotubular zone and disjunction commisure and incomplete Central koaptation leafets of the aortic valve.Annulo-aortic ectasia and connective tissue disease as Marfan syndrome, Jellersa-Danlos syndrome and Lois-Dic: Sinus Valsalva's extension, sinotubular zones and the fibrous ring mechanism connected with cystic medial necrosis. Interesting that the valve leaflets are not affected.

2. The reason of aortic insufficiency in these syndromes connected with the progressive expansion of all components except the aortic root valve leaflets, which often leads to the dissection of the aortic wall. Acute or chronic aortic dissection : when aortic root aneurism dissections there is an aortic insufficiency or sinotubular valve extension (a), or detachment commisure with prolapse of leaflets (b). In the absence of damage to the leaflets, the aortic wall and pathology of aortic root, often possible the replacement and reconstruction of the aortic valve [11-27].

Reconstruction-oriented functional classification of aortic insufficiency
(de Kerchove and El Khoury. Anatomy and pathophysiology of the VAJ DOI: 10.3978/j.issn.2225-319X.2012.12.05) (.)

Category	Type I — fibrous ring's dilation or cusp's fenestration in normal excursion				Type II Leaflets prolapse	Type III Limit of cusp's excursion
	Ia	Ib	Ic	Id		
Mechanism						
Repairing technique	Remodelling of CTC ascending aorta by prosthesis	valve-sparing surgery : reimplantation or remodeling with subcommutational annuplasty	subcommuta-tional annuplasty	replacement with xeno-pericardial patch	– free fimbria's plication – free fimbria's resuspensuion – triangural exsection	– parietal exsection – decalcination – using of patch
Postprimary	subcommuta-tional annuplasty		annuplasty of synotubular junction	subcommutati onal annuplasty	subcommutatio-nal annuplasty	subcommuta-tional annuplasty

In accordance with this approach, it is proposed:

1. In the case of ascending aorta, extending to CTC use replacement of ascending aorta and resuspension of CTC.

2. In the case of aneurism extending to sinus use remodeling procedure and subvalvular annuplasty or reimplantation valve to prosthesis of ascending aorta

3. In the case of ring dilation implement different types of annuplasty.

4.In the case of cusp perforation – reconstruction leaflets' autopericardium patch.

5. In the case of leaflets' prolapse:

a) reefing of free fimbria, it's resuspension, rehabilitate length prolapsing cusp by the use of pericardium;

b) sectoral exsection of excessive leaved tela.

6. In the case of leaflets' excursion limit:

a) use the method of triangular and parietal exsection shaving of free fimbria, fibrous incrassated fimbria for rehabilitate excursion , abolition commissural adherence;

b) rehabilitate length and integration shortened leaflets by use of autopericardium patch in order to assure leaflets' cooptation and regurgitation's liquidation

Aortic valve conservation's efficiency. The main idea of aortic valve conservation operations is the rehabilitation of functional anatomy of aortic root,because we often face the aortic root deformation without any structural and morphological changes on the part of valve leaflets [19, 20]. Aortic root reconsruction is more preferred than replacement , because there is no potential risk for complication as embolization of thrombus, prosthesis disfunction and endocarditis. The risk of early technical failures can be reduced through the regular usage intraoperative transoesophageal echocardiography and necessarily immediate interference until the moment of relocation to intensive cure unit.

The history of development valve conservational surgery and plastic surgery to aortic root. Corrigan first described aortic insuffiecency caused by the sinotubular junction's dilatation, without changes in valve leaflets. In 1913, Tuffier reported about the first aortic valve commissurotomy about his stenosis [23]. In 1956, Lewis published own plastic aortic valve technique and in 1985 Hapken reported about aortic stenosis's decalcification and plastic;at the first results of these plastic operations' were unsatisfactory, the effect was evanescent [22, 23].

In 1958, Taylor described the technique reducing the aortic insufficiency, which consisted circular saturation , coarctating and retracting the size of aortic ring and aortic aneurism [24]. In 1959 году, Starzl reported about the new technique of reducing aortic insufficiency through valve bicuspidization [25]. In 1960, Murphy described the technique of fibrous ring placation in the case of syphilitic aotic root damage and aortic insufficiency, which implements without artificial blood circulation. It is similar to the Hurwit's technique. (1960). In 1958, Garamella published his aortic insufficiency therapy theory through commissure resuspension. This successful method became the important period in treatment development and understanding of core's semilunar valve fuctions [29].

In 1968, Bentall and De Bono, in a two-page message, described one patient who had replaced the aortic root and the ascending aorta with a composite prosthesis consisting of a suction tube and a valve prosthesis, the coronary artery mouth was implanted into the wall of the conduit. Subsequently, this technique became the gold standard in surgery of an aneurysm of the ascending aorta and bundle [30]. In 1980 and 1983, Wolfe reported on a series of reconstructive surgeries - aortic valve resuspension - performed in acute aortic dissection. In his work from 1983, 48 patients were reported, resuspension was successfully performed in 35 of them [29,30]. Only in one case, 17 years after the operation, the patient needed a reoperation - prosthetic repair of the aortic valve. In 1986, Frater described and emphasized the anatomical and mechanical function of the sinotubular junction, noting that correction of the extended sinotubular junction is often sufficient to eliminate aortic valve failure, provided that the valve flaps and its fibrous ring are not dilated [31].

Development of modern methods of restoring the aortic valve in aneurysm and aortic dissection. The technique was first described by M. De Bakey in 1956. It is one of the main valve-preserving surgeries for an aneurysm of the ascending aorta with associated aortic insufficiency caused by dilated STS [32, 33]. The technique consists in resection of the ascending aorta at the level of the STS, followed by the imposition of proximal anastomosis at the level of CTC with a synthetic aortic prosthesis (figure 2). Restoring the normal diameter of the CTC leads to the closeness of the valves and the restoration of coaptation, which eliminates aortic insufficiency. Back in 1986, Frater described and emphasized the anatomical and mechanical function of the sinotubular junction, indicating that only the reduction of the expanded sino-tubular junction can be performed to correct aortic insufficiency, provided that the valve flaps and its fibrous ring are not dilated. This technique is still widely used, showing good results in the long-term, especially in patients with aortic aneurysms without delamination.

The aortic valve resuspension technique is used today to correct all types of stratification, involving the ascending aorta above the synthubular crest or involving the non-coronary valve and its prolapse (figure 3).

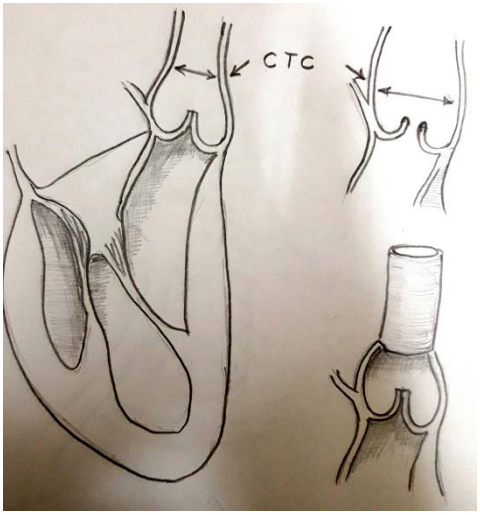


Figure 2 – Supracoronary prosthesis of the ascending aorta by Debakey

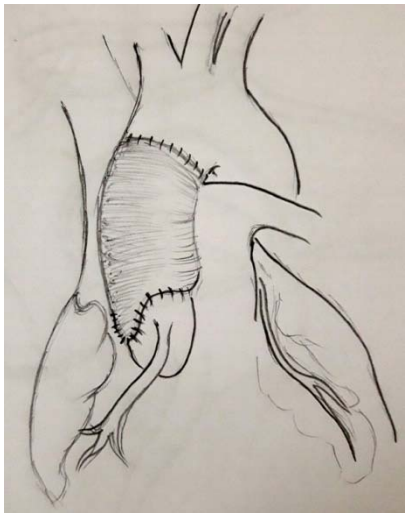


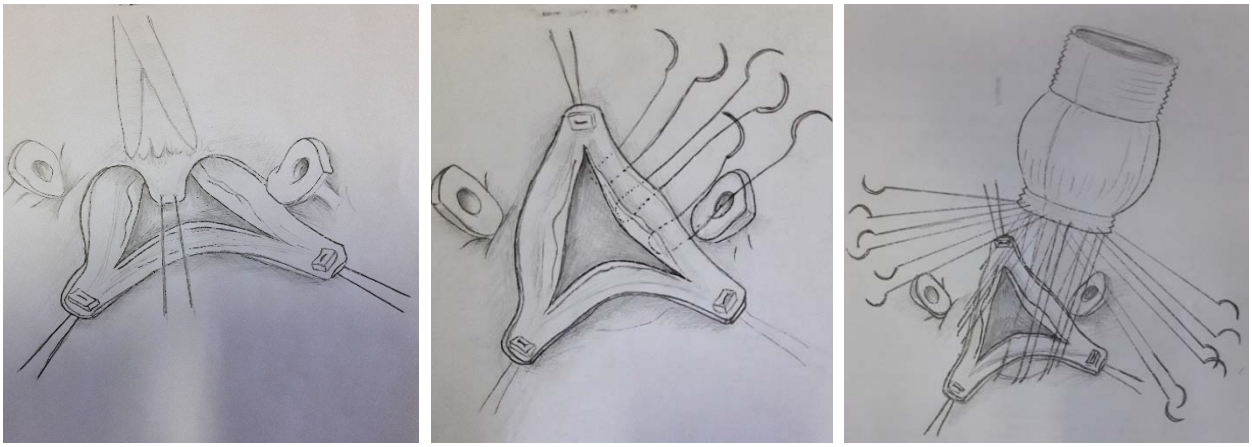
Figure 3 – Operation Wolfe

Surgery Wolfe, the non-coronary sinus (most often involved in the bundle) is excised and prosthetized by the tongue of the dacron prosthesis. The commissure posts of the valve are suspended in the Dacron tube, the biological glue is used to restore the exfoliated layers of the aorta, and also to seal the anastomoses of this reconstruction.

Aortic valve reimplantation, David and Feindel technique: TD 1. In 1992, David and Feindel reported a series of patients (n = 10) who underwent valve-conserving surgery for an aortic aneurysm with aortic insufficiency (34).

This technique is the purpose of this review and will be referred to as Tirone David 1 (TD1), consisting of a classic reimplantation of the aortic valve inside the dacronoptic prosthesis. To do this, aortic root resection was performed with the coronary arteries left on the sites and valve flaps on commissures with a 4-5 mm sinus wall site (figure 3, A, B). Dacron prosthesis was sutured to the base of the root of the aorta by stitches on the gaskets, the seams were placed below the valves of the AK so that they would pass through the fibrous skeleton of the base of the outlet part of the left ventricle (pseudo-ring). The valve commissures are sewn inside the Dacron tube in such a way, to achieve coaptation of the valves.

The operation is completed by sewing the coronary "pushes" to the neosinos and deposing the distal anastomosis (figure 4). Features of the reimplantation operation TD1 is a cylindrical reconstruction, reimplantation of the coronary arteries and maximum stabilization of the base of the root of the aorta (ring). In



a *b* *c*

Figure 4 – Technique Tirone David 1, sequentially shows the stages of the operation, first described in 1992

the original technique, there is no description of the specificity of the sinotubular compound. Hvass reports a change in this technique in such a way that the dacron prosthesis is stitched inside the root of the aorta to the base of the valve flaps (in the David method – vice versa); Such a method of reimplantation has disadvantages – the AK leaflet contacts with the dacron tissue, and a stable fixation of the fibrous valve ring is not created [35].

Resection of the aneurysm of the ascending aorta, the valves and columns of the commissure of the valve are cut out, leaving the sinus wall at the edge of 3-5 mm, for suturing. The mouths of the coronary arteries are cut out on the "buttons". The vascular (dacron) prosthesis is sewn to the distal part of the ascending aorta. These sutures are sewn the end of the selected dacron prosthesis. The prosthesis was fixed to the base of the root of the aorta. The posts of the commissure are suspended inside the prosthesis with a continuous suturing seam, like the implantation technique of the subcoronary homograft. Anastomoses are applied with coronary arteries and a distal interstitial anastomosis.

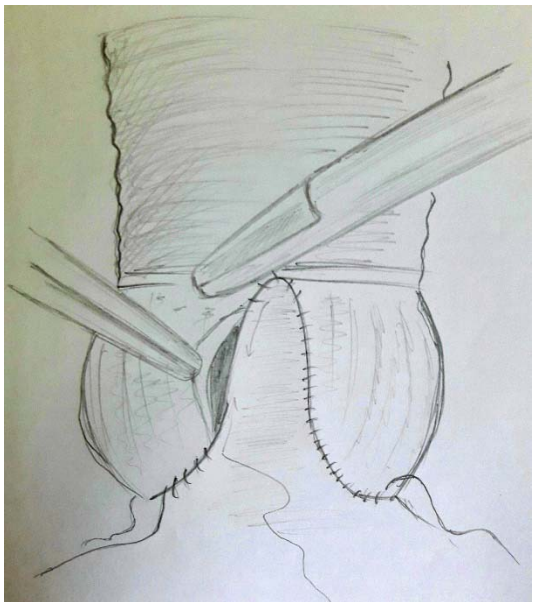


Figure 5 – The technique of remodeling the root of the aorta Yacoub, 1993

In 1993, Sarsam and Yacoub give a series of observations (10 patients) of surgical treatment of aortic insufficiency by a method called "remodeling of the aortic ring" [36].

The first version of the Yakub operation consisted of prosthetics of all three sinuses of Valsalva with reimplantation of the coronary arteries with the use of a dacron prosthesis truncated as a three-petal crown. The method involves reimplantation of the coronary arteries, but does not provide for the stabilization of the base of the aortic root and the determination of the size of the sinotubular junction (figure 5). In their technique, the authors emphasize the need to excise the sinuses of the aorta and the selection of a dacron tube of diameter equal to the diameter of the base of the root of the aorta. The incisions on the prosthesis are performed to increase the fixation height of the commissures of the valve. According to the name, a cylindrical reconstruction of the root of the aorta is performed, there is no specific narrowing at the site of the sinotubular junction, and there is no stabilization of the base of the aorta. Advantages of this method is technical simplicity, than with reimplantation, and allows more accurate resurrection of commissural racks. Subsequent modifications of the Yakub operation include a narrowing in the area of the sinotubular crest and the creation of swelling sinuses. Fig.5 Technique of remodeling Sarsam and Yacoub, 1993.

Reimplantation or remodeling. In 1995, David, Feindel and Boss reported the next step in the evolution of Tirone David's methodology. In the article "Reconstruction of the aortic valve with its insufficiency and aneurysm of the root of the aorta" [37], there are two fundamentally different recovery methods. The first was the reimplantation technique - TD1, which was used in patients with the expansion of sinotubular junctions, the destroyed or dilated sinus of Valsalva and with annuloaortal ectasia. An alter-

native technique, given immediately, was called "Tirone Davide-2" and described by the authors as a technique of "remodeling", was used in patients without annuloaortal ectasia, in most cases with Valsalva sinus deformity and the need for correction of the sinotubular junction. In this series, 45 patients were described, death was observed only in two cases. Nineteen patients were operated on by the reimplantation technique TD-1 and 26 by the TD-2 remodeling technique, the aortic valve was restored by reconstruction and prosthetics of all sinuses of the Valsalva coronary artery mouth was reimplanted into the prosthesis using the "push" technique. There was no strengthening or reduction of the "aortic ring", there was also no remodeling of the sinotubular junction (i.e., a decrease in diameter by 15% of the diameter of the outlet LV).

Reimplantation of aortic root with reconstruction of pseudo-sinuses: Seattle technique methodology. In 1995, Cochran and colleagues described a version of the operation of Tirone David, in which a Dacron tube with formed convex pseudo-sinuses was used, with the same valve retention technique [38]. In Seattle technique, the aortic valve is preserved and prosthetics of all sinuses of Valsalva is performed with reconstruction of neosinos using special techniques. The swelling of these neo-sinus prevents the contact of the leaf with the dacron tissue. In the normal aorta, the valves are protected by dynamic geometry (dilatation of the sinuses, lengthening of the valves and root of the aorta). This technique also stabilizes the "aortic ring" with the proximal suture of the valve flaps below, similar to the TD-1 technique, as well as a distal seam, above the flaps, designed to maximize fixation of the commissure racks. Cutting out the petals at the proximal end of the prosthesis is necessary for the formation of neo-sinuses.

The hybrid technique was suggested by van Son and co-authors, in which the enlarged aortic root is reduced and reconstructed (coronary arteries are cut out on the buttons) by wedge-shaped excision of the Valsalva sinus walls, and then the reimplantation of the restored aortic root into the dacron tube, with careful restoration of the height of the commissural racks; The distal suture between the prosthesis and the root of the aorta is superimposed, and then the coronary arteries are filed [39]. This mixed technique allows the reconstruction of sinuses, taking into account the control of the diameters of the sinotubular junction and the fibrous ring, which avoids the contact of the valve with the dacron prosthesis, but does not stabilize the base of the root of the aorta. Some authors leave the "cushions" of the aortic wall, theoretically to protect the valves from damage [40, 41].

In 1996, T. David described another version of his remodeling method (TD-2), which is used for annuloaortal ectasia (TD-3 technique). In this case, the aortic valve is reconstructed by prosthetics of all those Valsalva sinuses, the coronary arteries are reimplanted into the prosthesis (figures 6, 7).

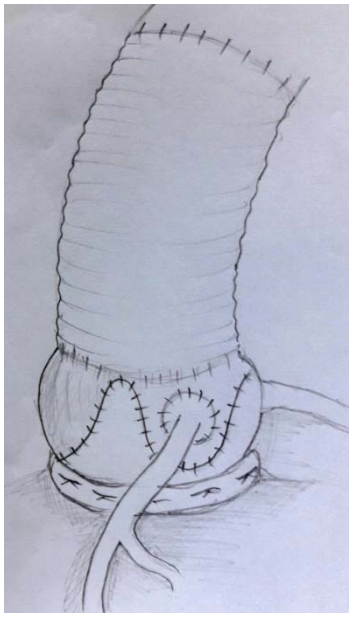


Figure 6 – Remodeling the root of the aorta with stabilization of the fibrous valve ring (TD-3)

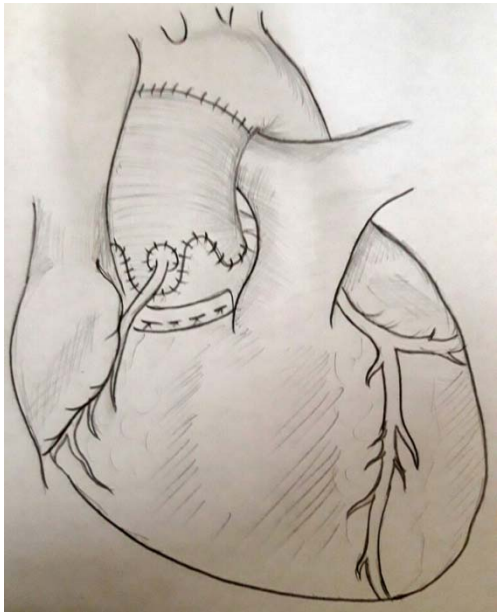


Figure 7 – Remodeling the root of the aorta with stabilization of the fibrous valve ring (TD-3)

The main difference of this technique is the strengthening of the root of the aorta and the fibrous ring with a teflon strip. This technique also provides an easier resuspension of the commissure columns, similar to the Yakub technique, and the remodeling of the sino-tubular junction does not depend on the size of the chosen prosthesis (figure 7 F-H). El Khoury and colleagues reported on this technique, but they limited themselves only to strengthening the aortic ring with significant dilatation; they also recommend the preservation of the intact sines of Valsalva [42].

Figures 6, 7. Remodeling Tirone David-3, 1996. Removal of the affected root of the aorta and the ascending aorta. The aorta intersects immediately above the sinotubular crest. The root of the aorta is mobilized to the very base, the coronary arteries are cut out on the sites. The aorta intersects immediately above the sinotubular crest. The root of the aorta is mobilized to the very base, the coronary arteries are cut out on the sites. The strip of Teflon is strengthened by the fibrosis of the exit region of the left ventricle, which gives stabilization of the base of the root of the aorta, especially necessary for annuloaortal ectasia. The diameter of the aortic ring (AA) was measured from it, the thickness of the aortic wall was subtracted, in order to determine the diameter of the necessary dacronoptic prosthesis. Aortic root remodeling method according to TD-3: Replacement of aortic root with a cut prosthesis. Adequate resuspension of the commissure. Reimplantation of coronary arteries into the corresponding sinuses.

Recovery of the sinotubular crest. This method consists of simple prosthetics of the ascending aorta with concomitant reconstruction (ie, constriction or reduction aortoplasty) of the sinotubular crest to restore normal co-aortic valves in the root of the aorta. As a separate method it can be used only with normal valves and sinuses of the Valsalva, and when the fibrous ring does not need reconstruction. Do not perform prosthetics of sinuses, and reimplantation of the coronary arteries. This operation does not carry in itself the strengthening and stabilization of the base of the root of the aorta, in essence this is the method of restoring the sinotubular junction, which Frater proposed [31]. Dr. David included this approach in his updated version of reimplantation (called Dr. Miller, as TD-4), in which he chooses a 4 mm dacron prosthesis larger than necessary and creates a sinotubular crest by its circular plication [43]. In the technique of reimplantation TD-5 or Miller-1, the dacron prosthesis is applied even 8 mm more than necessary, due to which synthetic pseudo-sinuses are formed [43, 44]. The "Jena" technique is hybrid, in which the reconstruction of the aortic root aneurysm is performed by plication and excision of a portion of the Valsalva sinus (U-shaped in the coronary and V-shaped in the non-coronary sinuses), and the sinotubular junction is reduced by the dacron prosthesis (26-28 mm). The advantage of this method is the preservation of native tissue that contacts the valve flaps, as well as the dynamic properties of the root of the aorta, but there is a risk of further dilatation of the reconstruction zone [45].

Remodeling of the root of the aorta with maximum stabilization, preservation of the valves and reconstruction of the sinotubular junction. Hopkins proposed his method of reconstructing the aortic valve, but in essence, this is one of the varieties of remodeling. In this technique, all sinuses are replaced with reimplantation of the coronary arteries (similar to the Yacoub or TD-2 methods). The base of the root of the aorta is strengthened by a circular seam applied below the valves (figure 8), this protects the aortic

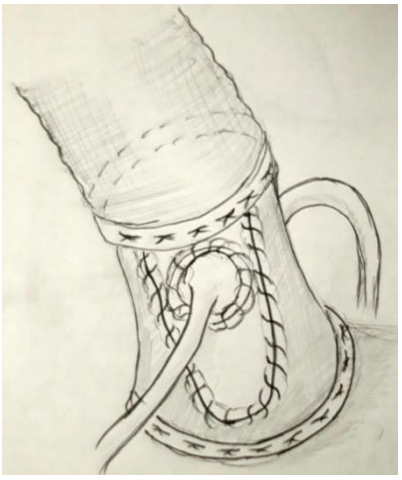


Figure 8

root enlargement in the following, and therefore is applicable to patients with congenital connective tissue defect, such as Marfan syndrome. As in Yacoub or TD-3 techniques, an uncomplicated procedure for resuspension of commissural racks is performed.

In this technique, the sinotubular junction is remodeled to ideal dimensions, i.e. 15% smaller than the diameter of the aortic ring, by selecting a dacron prosthesis smaller in diameter than the aortic ring, the sinuses slightly bulge outward, which is achieved by higher notches on the prosthesis, which are suspended by the commissure of the valve. The cuts on the prosthesis must be narrow to emphasize the place of the synthobular transition, then the narrowing of this place is made by the Teflon strip. By narrowing the sinotubular junction, the functional flows described by Da Vinci that prevent the valves from contacting the dacronoptic prosthesis are retained. This reconstruction covers all components of the root of the aorta, while preserving the natural valves of the aortic valve.

Method of Hopkins-1. Narrowing strips of Teflon are applied circularly after excision of the pathologically altered aorta, and after the mobilization of its root. The proximal band is fixed by a series of mattress sutures, below the flaps. Commissures are kept with a site of surrounding tissue 3-5 mm. The notches in the proximal end of the prosthesis are made a bit longer, which will allow the commissure to be sutured even higher, and due to this, the neosinus will be convex. A proximal suture is applied at the base of the root of the aorta, the sinuses are replaced by the dacron prosthesis. Reimplantation of coronary mouths, the formation of constriction in the place where the commissure of the valve is sewn.

Reimplantation of the root of the aorta into the prosthesis. Method of Florida Sleeve, described by R. Hess in 2005. The ascending aorta crosses 1 cm above the sinotubular junction, the root of the aorta is allocated along the circumference to the level of the aortocastular contact. The diameter of the fibrous ring of the aortic valve is determined by using standard-sized meters. The distance from the base of the root of the aorta to the coronary arteries and the sinotubular crest for each commissure is also measured, which is necessary for the preparation of the prosthesis. The choice of prosthesis is performed at a rate of 4-5 mm larger than the measured diameter of the fibrous ring. The height of the skirt of the prosthesis should correspond to the measured height for each of the commissures, all commissures must be at the level of the sino-tubular crest of the prosthesis. Then, the coronary arteries are located on the prosthesis, after which vertical slots are made in the indicated positions in the form of a "keyhole". The length of the slits corresponds to the measured distance from the root of the aortic root to the lower part of the coronary artery. At the location of the coronary artery mouths, round holes are made. The next step is to place subannular U-shaped seams with a 3/0 woven with Teflon liners, placing them horizontally in a circle 1-2 mm below the aortic valve flaps from the inside out, so that the gaskets do not touch the cusps (figures 9, 10). In the area between the non-coronary and right coronary sinuses, seams must be placed along the contour of the valves to avoid complications from the side of the conductive system and the membrane part of the interventricular septum [46-48].

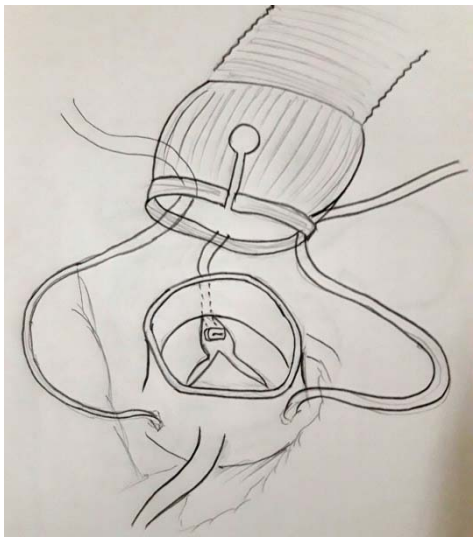


Figure 9 – Method of Florida Sleeve

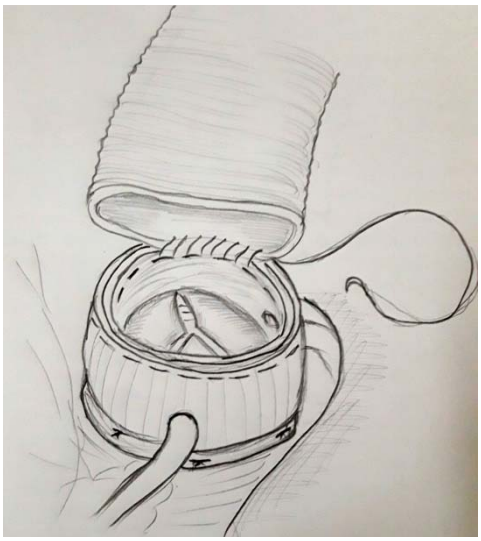


Figure 10 – Method of Florida Sleeve

Cases of failure of reimplantation and remodeling of the aortic valve. Unsuccessful valve-preserving surgeries included procedures in which there is no adequate stabilization of the aortic base, and which in the long-term period lead to dilatation of the aortic ring [49]. Part of the output aortic tract forms a functional ring - in fact the bases of the interlobic triangles are below the fixation of the valves and is part of the ventricular hemodynamics [9, 10]. Proponents of reimplantation technology suggest that the stabilization of the aortic ring, especially in connective tissue diseases, is an important point. Remodeling methods leave these triangles intact, which should positively influence the surgery of the preserved flaps and the duration of reconstruction, since some elements of the dynamic expansion of the aortic root are preserved. Implantation methods support or fix these interlobular triangles.

The theoretical reason for the accelerated degeneration of the valves is systolic contact with the prosthetic wall, due to the lack of sinus expansion. Attempts to unsuccessfully reconstruct the root of the aorta with a ring size of 25-27 mm were attributed to the injury of the valves due to restraint in the prosthesis [11, 50-52]. Such complications can be during reimplantation in a cylindrical prosthesis or during remodeling, when a smaller prosthesis size is used. To avoid such problems, aortic prostheses with artificial sinuses of Valsalva are being developed [53].

Pethig and colleagues, using an echocardiographic study, determined that the level at which the leaflet coaptation occurs is an important factor in the long-term reconstruction. They divided all patients into three groups, depending on the level of coaptation: A – coaptation of the valves 2 mm above the plane of the aortic ring; B – at the level of the plane of the base of the ring; C – below the level of the base of the aortic ring. There was no regurgitation in group A (n = 56); in group B, severe AS occurred in 2 patients (n = 11); all patients from group C had AK deficiency (54).

Early methods of remodeling the root of the aorta (Yacoub, David-2) were aimed at prosthetics of pathologically altered sinuses of the Valsalva and preservation of the dynamic properties of the aorto-ventricular zone. However, in patients with connective tissue diseases, long-term complications associated with dilatation of unfortified aortic root components may develop, so this method developed further in the direction of greater stabilization of the base of the aorta and sinotubular zone.

Dr. David compared his reimplantation technique with developing remodeling methods in patients with an aortic root aneurysm and found some disparity in the long-term nature of these techniques. While the 8-10 year survival was excellent, his study showed the best results (ie freedom from developing moderate and severe AK insufficiency) for reimplantation technique [49]. He correctly noted that without strengthening, the root of the aorta can expand after many of the remodeling techniques. While, the technique of reimplantation theoretically has disadvantages - the contact of the cusps with synthetic "sinuses", in reality this is not a serious problem [49, 53, 55]. In addition, dacron prostheses with convex sinuses became available today [53, 56-58].

The extended service life of the reconstructed cusps can be achieved surgically by either Seattle technique or the use of prostheses proposed by Zehr [59] or the described David technique [49] or by using a Valsalva sinus prosthesis in combination with a smaller diameter tube, as in the Mayo Clinic [50]. However, the mechanism of the development of aortic insufficiency in the late period after the remodeling surgery is not entirely clear, most likely because of insufficient strengthening of the residual fibromuscular components of the complex - the root of the aorta, which is characteristic for this procedure.

In addition, problems can arise due to brittleness and postoperative degeneration and subsequent thinning of the cusps.

Choosing the size of the prosthesis for reimplantation or remodeling. A great attention was paid to the choice of the size of the prosthesis for various methods of reconstruction of the root of the aorta. The technique of reimplantation has the advantage of complete fixation of the whole complex of the root of the aorta and placement of the valve in the dacron prosthesis, however, its choice differs from the process of remodeling. Reimplantation requires the choice of a sufficiently large prosthesis, that would not increase the area of coaptation of the cusps. David stressed that the sinotubular junction can be formed either by narrowing the prosthesis or by sewing a smaller diameter prosthesis at the level of this junction if necessary; the procedure of reimplantation in general requires a fairly wide prosthesis (30-34 mm), which ideally corresponds to the size of the non-enlarged aortic ring. To select the size of the prosthesis in remodeling surgeries, several methods are described [3].

According to the method of M.Yacoub, in order to determine the required value commissures are stretched vertically, the position of the cusps is determined and their ability to coapt without prolapse; the necessary diameter is equal to the distance between the vertices of the commissure or one third of the aortic circle at the level of the sinotubular junction. David suggested first to normalize the aortic ring, and then choose a prosthesis, given that the diameter of the sinotubular transition is less than 15% of the aortic ring. This corresponds to the Kunzelman morphometry [2]. The Yakub group suggests that you also measure the distances between commissures, with the maximum coaptation of the wings, and then calculate the diameter of the prosthesis. K.Morishita et al. Have suggested to use the following formula:

$$d = 2 / \sqrt{3} \times id,$$

where *d* is the required diameter of the prosthesis, *id* is the distance between the commissure vertices.

David T. David's opinion is that the approximate diameter of the prosthesis is equal to the length of the free edge of the aortic valve leaf, minus 10% of the figure, but there is absolutely no exact formula for calculating the size of the prosthesis and the solution depends on the surgeon's experience. In valve-preserving surgeries with the reconstruction of the sinotubular ridge, the required diameter of the prosthesis is twice the height of the aortic valve leaflet. Dr. David noted that determining the size of the root of the aorta and the sinotubular junction is more an art than an exact science [60]. In his hands, the choice of prosthesis for reimplantation differs from that for remodeling [49]. The concept of choosing a prosthesis for reimplantation is based on the external diameter of the root of the aorta (internal diameter + wall thickness), while in remodeling - on the inner diameter. When remodeling, David is based on the size of the valves and does not recommend the use of prostheses less than 30 mm to avoid restriction of the sines and subsequent damage to the valves [49, 60, 66]. For remodeling, we described the following procedure. After excision of the sinuses, the true diameter of the aortoventricular connection is made with the help of the Gegar dilator. Horizontal horizontal mattresses are applied to the top of each commissural column, which are then stretched up to the appropriate diameter of the sinotubular junction, while a hydrodynamic coaptation test is performed. This diameter of the sinotubular zone usually corresponds to the internal diameter of the exit aortic tract, which was measured by Gegar. If the coaptation area of the semilunar valves seems insufficient, then a smaller diameter prosthesis should be selected. This is a very simple method (similar to David's artistic approach), where the narrowing of the prosthesis is combined with a strip of Teflon in the area of the sinotubular junction and an accurate selection of the commissure fixation height, which gives a reliable result of the reconstruction of the sinotubular junction zone without deformation of the sinuses.

The height of the commissure between the non-coronary-left coronary is measured to determine the size of the graft from the line connecting the nadir of two adjacent flaps (the base of the triangle between the rows) to the top of the commissure. This measurement corresponds to the size of the selected transplant; B. In the Gelweave Valsalva ™ transplant (Vascutek Ltd, Terumo, Renfrewshire, Scotland), the height of the sinus part is equal to its diameter, which corresponds to the labeled size.

Results of reconstruction of the root of the aorta with preservation or restoration of the aortic valve. The level of surgery mortality varies from 0% to 6% [70,71] with a survival rate in 7 years 72-78% 8% [71, 72]. Patients with an aneurysm of the ascending aorta have a survival rate lower than those with an aortic root aneurysm, about 36% survive in the 8-year period [49]. This is a low survival rate, probably associated with the elderly age of patients with an aneurysm of VA, as well as concomitant vascular pathology. Resurgeries after aortic valve replacement for 7-8 years have a low frequency and according to many authors the freedom from this kind of rsurgeries is 90-97% [68, 71, 73]. Moderate aortic insufficiency is a rarity, especially during the first 2 years after surgery. However, the expressed AS is often close to 6%, although in some reports it reaches a level of up to 37% [34, 55, 73-78]. In addition to good survival rates in patients who underwent reconstruction of the root of the aorta, two-thirds of those observed are free from the risk of developing a moderate and severe degree of aortic valve failure within 8 years after surgery (49). David notes the superiority of reimplantation techniques (over remodeling) with a low risk of aortic insufficiency in the future; the average and severe AS was found in 10% for the 8-year period, while, when remodeling, this index was 45% [78]. The Hanover group achieved the same success in reimplantation of the aortic root, hospital mortality was 3.8%, and 4% were reopera- ted for aortic insufficiency [73]. Intraoperative transesophageal echocardiographic evaluation is important in determining

the long-term duration of the surgery [55]. Later complications (aortic insufficiency) in patients with connective tissue diseases are revealed more often, in methods where there is no maximum stabilization of the root of the aorta root ("ring") [60, 66-69,78-80].

Patients with an aneurysm of the root of the aorta and an intact root complex diseases, in which the reconstruction consisted of normalizing the diameter of the sinotubular zone, immediate and intermediate functional results are very good, and more than two-thirds of patients are free from development of the AS in the period of 8-10 years after surgery; however, the overall survival of these patients is relatively low, and only a third remains alive by the year 8, and possibly concomitant vascular disease and the age of the patients [49].

To compare the reconstruction technique, Dr. Gott and colleagues, examined the results of prosthetics in 235 patients with Marfan syndrome, 232 of whom underwent Bental's surgery and prosthetic aortic root replacement. In this group, there were no deaths in 30 days, 85% of these patients were alive at the time of publication of this article, and freedom from re-surgery for 20 years was 74% [81]. In the Japanese study, corrugated conduit was used, the operative mortality was 8.3%, and the actuary survival rate by the year 5 was 82.7% 4.8% [82].

Edwards and colleagues, using the National STS Cardiac Surgery database, determined the rates of operative mortality for isolated aortic valve replacement-4%; for patients with planned prosthetics AK-3,3% [83]. These data suggest that in selective patients, planned valve-saving surgeries are currently performed in most centers with a death rate approaching or better than for isolated aortic valve replacement.

Conclusion. The progress of these methods occurred after understanding the functional anatomy of the aortic root complex. Preliminary results of such surgeries support interest in their use, but an ideal and safe reconstruction technique, especially for connective tissue diseases, for example Marfan syndrome, should be determined after a longer period of postoperative follow-up (43). Today, there are many methods of reconstructing the root of the aorta, some of the original techniques have been replaced or modified by the same authors. The surgeon should consider the root of the aorta as a complex of elements, and strive to optimize its functional anatomy in each patient individually. The development of new types of prostheses also facilitates this task. Knowledge of the specific anatomy and history of the disease of each patient should help in the successful reconstruction of the aortic root complex with lasting effect and low lethality.

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ҚОЛҚА ҚАҚПАҚШАСЫНЫҢ ЖЕТІСПЕУШІЛІГІМЕН ҚОСАРЛАНҒАН ҚОЛҚА ТҮБІРІНІҢ АНЕВРИЗМАСЫ КЕЗІНДЕГІ РЕКОНСТРУКТИВТІ ОПЕРАЦИЯЛАР

Аннотация. Қазіргі танда қолқа түбірінің аневризмасы кезіндегі реконструктивті операцияларға кардиохирургия саласында қызу талқылануда. Соңғы отыз жылдықта қолқа түбірінің аневризмасы кезінде жасалатын операциялардың бірнеше әдіс-тәсілдері ұсынылған. Олар бір-бірінен жасалу техникасы және түзету жүргізілетін анатомиялық аймағына байланысты ерекшеленеді. Бұл әдіс-тәсілдердің мақсаты қызметі сақталған жармаларды сақтап, қолқа түбірінің басқа бөліктерінің тұқтандыру болып табылады. Бұл шаралардың беріктігі мен нәтижесінің ұзақ сақталуының маңыздылығы антикоагулянтарды қолдануда қажеттіліктің болмауында жатыр. Хирургиялық түзетудің әдіс-тәсілдерін таңдауда осы уақытқа дейін пікірталас бар, және әр жағдайда таңдау хирургқа қалады. Бұл шолуда қолқа түбірінің реконструкцивті операцияларын таңдауды жүйелі түрде қаралған және операциялардың графикалық иллюстрациялармен берілген. Операциялардың иллюстрациялармен суреттелуі хирургтың әдіс-тәсілдерді таңдауын жеңілдетеді.

Түйін сөздер: аорта түбірінің аневризмасы, қолқа қақпақшасының жеткіліксіздігі, аорта түбірінің реконструкциясы.

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РЕКОНСТРУКТИВНЫЕ ОПЕРАЦИИ ПРИ АНЕВРИЗМАХ КОРНЯ АОРТЫ С АОРТАЛЬНОЙ НЕДОСТАТОЧНОСТЬЮ

Аннотация. Реконструктивная хирургия корня аорты представляет большой интерес в области кардиохирургии. За последние три десятилетия был предложен ряд методик для коррекций аневризмы корня аорты с аортальной недостаточностью, различающиеся техническим выполнением и анатомической областью коррекции. Целью данных процедур является сохранение функционирующих створок и стабилизация других компонентов корня аорты. Надежный и длительный эффект таких вмешательств особенно важен ввиду отсутствия необходимости приема антикоагулянтов. Выбор метода хирургической коррекции до сих пор остается дискутабельным, и в каждом случае выбор остается за оперирующим хирургом. В данном обзоре описаны методы реконструкции корня аорты с систематическим подходом в выборе хирургического метода коррекции с графическими иллюстрациями операции. Описание операции с иллюстрациями способствуют упрощению выбора хирургического метода в каждом индивидуальном случае для хирурга.

Ключевые слова: аневризма корня аорты, аортальная недостаточность, реконструкция корня аорты.

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RECONSTRUCTIVE OPERATIONS IN AORTIC ROOF ANEURYMS WITH AORTAL INSUFFICIENCY

Abstract. Reconstructive surgery of the ao36tic root is of great interest in the field of cardiac surgery. Over the past three decades, a number of techniques, differing in technical performance and the anatomical area of the correction, have been proposed for the correction of aortic root aneurysm with aortic valve insufficiency. The purpose of these procedures is to maintain the functioning of the cusps and stabilize other components of the aortic root. Reliable and long-lasting effect of such interventions is particularly important due to the lack of necessity of taking anticoagulants. The choice of the method of surgical correction still remains controversial and in each case the surgeon is the one to make the decision. This review describes the methods of the aorta root reconstruction with a systematic approach of choosing a surgical correction method, accompanied by illustrations of the operations. The description of the procedures, followed by illustrations, facilitates the selection of the surgical method in each individual case.

Keywords: aortic root aneurysm, aortic insufficiency, reconstruction of aortic root.

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Аннотация. Реконструктивная хирургия корня аорты представляет большой интерес в области кардиохирургии. За последние три десятилетия был предложен ряд методик для коррекций аневризмы корня аорты с аортальной недостаточностью, различающиеся техническим выполнением и анатомической областью коррекции. Целью данных процедур является сохранение функционирующих створок и стабилизация других компонентов корня аорты. Надежный и длительный эффект таких вмешательств особенно важен ввиду отсутствия необходимости приема антикоагулянтов. Выбор метода хирургической коррекции до сих

пор остается дискутабельным, и в каждом случае выбор остается за оперирующим хирургом. В данном обзоре описаны методы реконструкции корня аорты с систематическим подходом в выборе хирургического метода коррекции с графическими иллюстрациями операции. Описание операции с иллюстрациями способствуют упрощению выбора хирургического метода в каждом индивидуальном случае для хирурга.

Ключевые слова: аневризма корня аорты, аортальная недостаточность, реконструкция корня аорты.

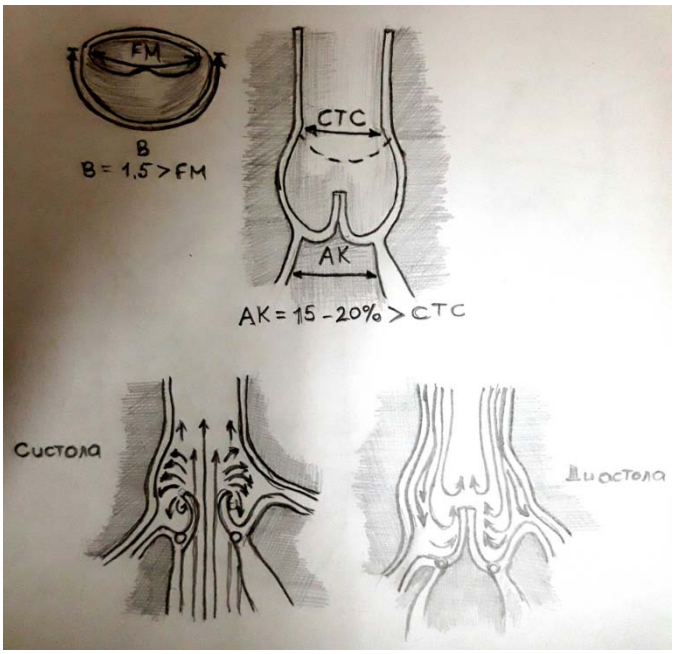
Введение. Большинство авторов считает, что под аневризмами аорты следует понимать местное или распространенное увеличение её диаметра в 1.5 раза (Svensson L.G., 1997, Покровский А.В. 1979, Белов Ю.В. 2011). Во многих случаях аневризму корня аорты сопровождает недостаточность аортального клапана. Наиболее частыми механизмами аортальной регургитации при аневризме аорты являются, аннуло-аортальная эктазия, расширение синотубулярного соединения и аневризма синусов Вальсальвы [84].

За последние два десятилетия было предложено ряд методик для коррекции аортальной недостаточности, различающиеся техническим выполнением и анатомической областью корня аорты. Целью данных процедур является сохранение функционирующих створок путем пластики клапана, протезирование или стабилизации других компонентов корня аорты [1]. Надежный и длительный эффект таких вмешательств особенно выгоден, в виду отсутствия в необходимости приема антикоагулянтов.

Целью данной статьи является обзор эволюции методов операции, определение показании к применению различных методик, основанных на восстановлении функциональной анатомии комплекса – «корень аоры».

Функциональная анатомия комплекса корня аорты и аортального клапана. В 1994 году, Kunzelman с соавторами опубликовали важную работу с описанием анатомии корня аорты и соотношение в нем аортального клапана [2]. Важной находкой статьи явилось то, что диаметр корня аорты на уровне середины синусов рассматривался как 100%, а диаметр на уровне синусного гребня составил 81% от этого размера и диаметр нормального основания корня аорты оставил 97% от первого показателя. Иными словами, диаметр синотубулярного гребня составляет приблизительно 85% от диаметра аортального кольца в основании корня. Этот количественный анализ анатомии корня аорты подтверждает теорию Да Винчи об вихревых потоках, созданными синотубулярным гребнем (рисунок 1). Завихряющиеся потоки жидкости, возникающие между краями створок клапана и стенкой аорты создают два эффекта:

1. В фазу открытия эти потоки предотвращают контакт лепестков клапана со стенкой аорты.
2. В фазу закрытия потоки инициируют закрытие створок.



Закрытие аортального клапана
и распределение потоков

AA = 15 – 20 % > STJ

Рисунок 1 – Статическая и функциональная анатомия корня аорты.
Диаметр синотубулярного соединения (STJ) меньше основания корня аорты (аортального кольца – AA) примерно на 15%

Второй эффект, описанный авторами, вероятно, даже более сложен, чем предложенный, – поток жидкости вызывает закрытие клапана в последний момент систолы, а вихревые потоки защищают створки от перегиба и способствуют более гладкому и синхронному их закрытию.

Динамическая анатомия корня аорты. Динамическая анатомия корня аорты была описана для понимания механизма снижения стресса на створки и таким образом, избежания износа и возможной структурной дисфункции клапана [3-10]. Группа из Стенфорда использовала радиоактивные маркеры на модели корня аорты у овцы, заметив при этом ряд сложных асимметричных деформаций в течение сердечного цикла, с вовлечением атриовентрикулярной зоны (предполагаемого аортального кольца) и синотубулярного соединения, а также удлинение, компрессию, расширение и разгибание корня аорты [3]. Четырехмерное исследование корня аорты, проведенное Lansac и соавт., подверждает что расширение корня аорты начинается в его основании в фазу изоволемического сокращения и отсюда распространяется на комиссуры клапана и в конечном счете – синотубулярное соединение [4]. Максимального расширения корень аорты достигает в первой трети систолы, приближаясь к цилиндрической форме, затем к середине диастолы идет уменьшение в объеме, и корень приобретает форму усеченного конуса. Расширение корня аорты на 39% и на 63% в комиссуральной области, дает улучшение оттока крови в систолу, а так же делает работу левого желудочка более эффективной.

Работы Dagum и Lansac описывают важность межлепестковых треугольников, описанных в работах Anderson и соавт., которые четко определяют аортальное кольцо, как действующую суб-аортальную структуру, состоящую из оснований 3-х межлепестковых треугольников, похожее на зубчатую линию прикрепления створок аортального клапана [3,4,9,10]. Основание корня аорты имеет свойство расширяться соответственно кинетики желудочка. Вверх корень аорты расширяется за счет расхождения межлепестковых треугольников, однако синусы и синотубулярный гребень максимально расширяются в конце систолы. Кроме того, этот точный динамический цикл расширения корня аорты, следует определенной хронологии, также влияя на позицию и степень расхождения оснований по которым прикреплены створки клапана, как и было впервые описано Thubricar [10] (рисунок 1). Все клапансохраняющие методики в разной степени меняют эту динамическую геометрию корня аорты.

Заболевания, при которых возможны клапансберегающие операции.

В основном, все эти реконструктивные операции применимы только в тех случаях, когда аортальная недостаточность обусловлена следующими поражениями (таблица):

1. Расширение корня аорты вторично по отношению к аневризме восходящей аорты: механизм аортальной недостаточности связан с расширением синотубулярной зоны и расхождением комиссур и неполной центральной коаптацией лепестков аортального клапана.

2. Аннуло-аортальная эктазия и заболевания соединительной ткани типа синдрома Марфана, Эллерса-Данло и Лойса-Дитца: механизм расширения синусов Вальсальвы, синотубулярной зоны и фиброзного кольца связанны с кистозным медионекрозом. Интересно, что створки клапана при этом остаются не пораженными. Причина аортальной недостаточности при таких синдромах связанна с прогрессивным расширением всех компонентов корня аорты кроме створок клапана, что часто приводит к расслоению аортальной стенки.

Расслоение аорты острое или хроническое: при расслаивающей аневризме корня аорты имеет место аортальная недостаточность вследствие либо расширения синотубулярного гребня (а), либо отслойка комиссур с пролапсом створок (б). При отсутствии повреждения створок, аортальной стенки и патологии корня аорты, часто возможна пластика и реконструкция аортального клапана [11-27].

В соответствии с данным подходом предлагается:

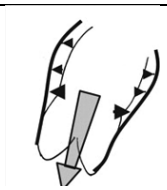
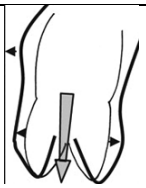
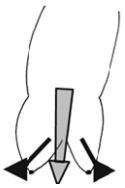
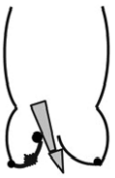
1. При аневризмах восходящей аорты, распространяющихся на СТС, выполнять протезирование восходящей аорты и ресуспензию СТС.

2. При распространении аневризмы на синусы использовать процедуры ремоделирования и субвальвулярной аннулопластики либо выполнение реимплантации клапана в протез восходящей аорты.

3. При дилатации кольца – выполнять различного рода аннулопластику.

4. При перфорации створок – реконструкции створок заплаты из аутоперикарда.

Реконструкция-ориентированная функциональная классификация аортальной недостаточности
(de Kerchove and El Khoury. Anatomy and pathophysiology of the VAJ DOI: 10.3978/j.issn.2225-319X.2012.12.05) (.)

Класс	Тип I – дилатация фиброзного кольца или фенестрация створки при нормальной подвижности створки				Тип II Пролапс створок	Тип III Ограничение подвижности створки
	Ia	Ib	Ic	Id		
Механизм						
Техника реконструкции (Первичная)	Ремоделирование СТС протезом восходящей аорты	Клапаносберегающие операция: реимплантация либо ремоделирование с субкоммиссуральной аннулопластикой	Субкоммиссуральная аннулопластика	Пластика с заплатой из ауто - из ксеноперикарда	– пликация свободного края – ресуспензия свободного края – триангулярная резекция	– париетальная резекция – декальцинация – использование заплаты
Вторичная	Субкоммиссур аннулопластика		Аннулопластика синотубулярного соединения	Субкоммиссур. аннулопластика	Субкоммиссур. аннулопластика	Субкоммиссур. аннулопластика

5. При пролапсе створок:

a) использовать пликацию свободного края, его ресуспензию, восстанавливать длину пролабирующей створки при помощи перикарда;

b) выполнять секторальную резекцию избыточной
створчатой ткани.

6. При ограничении (рестрикции) подвижности створок:

a) применять методики треугольной и париетальной резекции «выбривание» свободного края (shaving) фиброзно утолщенных краев створок для восстановления подвижности, устранения комиссурального их сращения;

b) восстанавливать длину и целостность укороченных створок при помощи заплат из аутоперикарда для обеспечения кооптации створок и ликвидации регургитации.

Рациональность сохранения аортального клапана. Основной идеей клапансберегающих операций является восстановление функциональной анатомии корня аорты, так как мы часто встречаем деформацию корня аорты без каких-либо структурных и морфологических изменений со стороны створок клапан. Реконструкция корня аорты более предпочтительна, чем протезирование, так как исчезает потенциальный риск таких осложнений как, тромбоэмболии, дисфункции протеза и эндокардит. Риск ранних технических неудач может быть снижен путем рутинного применения интраоперационной чрезпищеводной ЭХОКГ и по необходимости немедленного вмешательства до момента перевода в реанимацию [19, 20].

История развития клапансохраняющей хирургии и реконструктивных операций на корне аорты. В 1832 году, Corrigan впервые описал аортальную недостаточность, обусловленную дилятацией синотубулярного соединения, причем створки клапана не были изменены. В 1913 году, Tuffier сообщает о первой комиссуротомии аортального клапана, выполненой по поводу его стеноза [23]. В 1956 году, Lewis публикует свою технику пластики аортального клапана, и в 1958 году, Hapken сообщает об декальцинации и пластике аортального стеноза; поначалу результаты таких пластических операций были неудовлетворительными, эффект – непродолжительным [22, 23]. В 1958 году, Taylor описал технику устранения аортальной недостаточности, которая состояла в наложении циркулярного шва, суживающего и уменьшающего размер аортального кольца и аневризмы аорты [24]. В 1959 году, Starzl сообщил о новой технике для лечения аортальной недостаточности путем бикуспидализации клапана [25]. В 1960 году, Murphy описал технику

пликации фиброзного кольца при сифилитическом поражении корня аорты и аортальной недостаточности, которая выполняется без искусственного кровообращения, она схожа с техникой, предложенной Hurwitt (1960). В 1958 году Garamella опубликовал свою концепцию лечения аортальной недостаточности путем ресуспензии (подвешивания) комиссур. Эта успешная методика явилась важным этапом развития лечения и понимания функции полулунных клапанов сердца [29].

В 1968 году, Bentall и De Bono в сообщении из двух страниц, описали одного пациента, которому было выполнено замещение корня аорты и восходящей аорты составным протезом, состоящим из сосудистой трубки и клапанного протеза, устья коронарных артерий имплантировались в стенку кондуита. Впоследствии эта методика стала золотым стандартом в хирургии аневризм восходящей аорты и расслоения [30]. В 1980 и 1983 годах, Wolfe сообщил о серии реконструктивных операций – ресуспензии аортального клапана – выполненных при остром расслоении аорты. В его работе от 1983 года, сообщается о 48 больных, ресуспензия была успешно выполнена у 35 из них [29, 30]. Только в одном случае, через 17 лет после операции, больному понадобилась реоперация – протезирование аортального клапана.

В 1986 году, Frater описал и подчеркнул анатомическую и механическую функцию синотубулярного соединения, отметив при этом, что коррекция расширенного синотубулярного соединения бывает часто достаточной для ликвидации недостаточности аортального клапана, при условии, что створки клапана и его фиброзное кольцо не дилятированы [31].

Развитие современных методов восстановления аортального клапана при аневризме и расслоение аорты. Методика впервые описана M. De Bakey в 1956 году. Является одной из основных клапаносохраняющих операций при аневризме восходящего отдела аорты с сопутствующей аортальной недостаточностью, вызванной дилатацией СТС [32, 33]. Методика заключается в резекции восходящего отдела аорты на уровне СТС с последующим наложением проксимального анастомоза на уровне СТС с синтетическим протезом аорты (рисунок 2). Восстановление нормального диаметра СТС приводит к сближению створок и восстановлению коаптации, что устраняет аортальную недостаточность. Еще в 1986 году, Frater описал и подчеркнул анатомическую и механическую функцию синотубулярного соединения, указав на то, что для коррекции аортальной недостаточности может быть выполнена только редукция расширенного синотубулярного соединения, при условии, что створки клапана и его фиброзное кольцо не дилатированы. Данная методика до сих пор широко используется, показывая хорошие результаты в отдаленном периоде, особенно у пациентов при аневризмах аорты без расслоения.

Методика ресуспензии аортального клапана сегодня применяется при коррекции всех видов расслоения, в которое вовлечена восходящая аорта выше синтубулярного гребня или с вовлечением некоронарной створки и её пролапсом (рисунок 3).

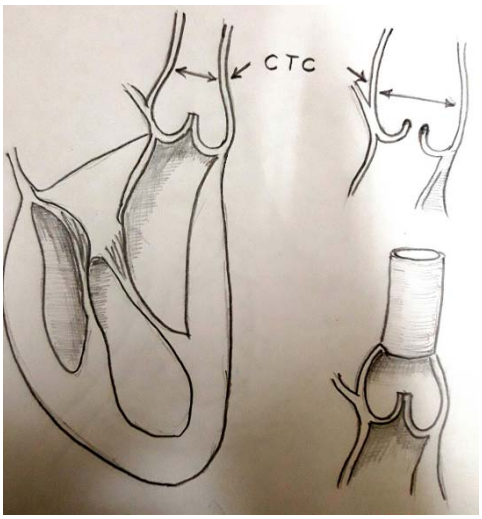


Рисунок 2 – Супракоронарное протезирование восходящегоотдела аорты по De Bakey

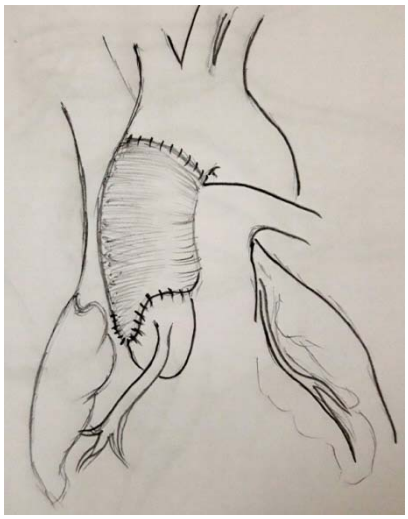
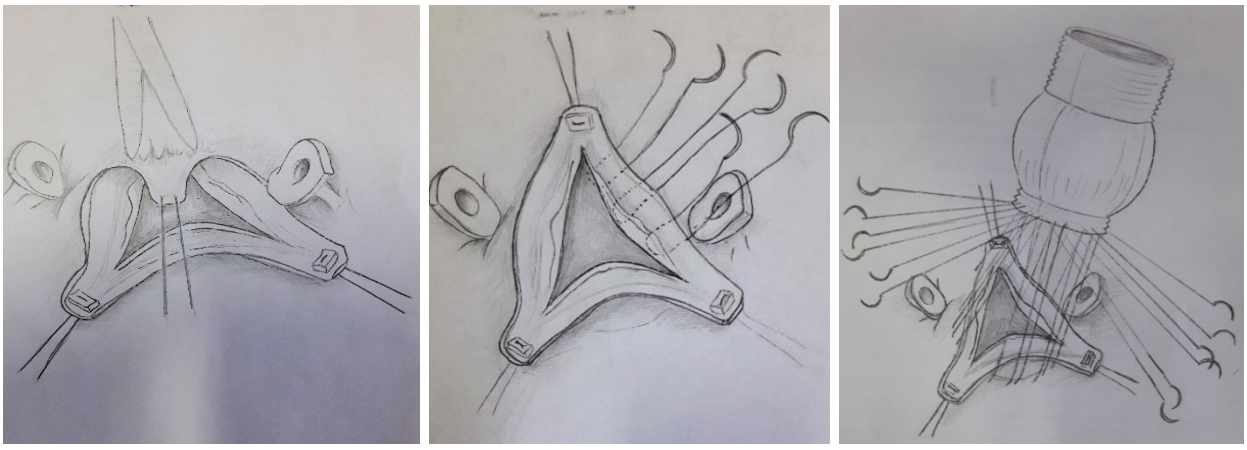


Рисунок 3 – Операция Wolfe

Операция Wolfe, некоронарный синус (чаще всего вовлеченный в расслоение) иссекается и протезируется язычком дакронового протеза. Стойки комиссур клапана подвешиваются в дакроновую трубку, биологический клей применяется для восстановления расслоенных слоев аорты, а также для герметизации анастомозов этой реконструкции

Реимплантация аортального клапана, техника David и Feindel: TD 1. В 1992 году, David и Feindel сообщили о серии пациентов (n = 10), которым выполняли клапансохраняющие операции по поводу аневризмы восходящей аорты с аортальной недостаточностью (34). Данная методика является целью этого обзора и будет обозначена как Tirone David 1 (TD1), состояла из классической реимплантации аортального клапана внутрь дакронового протеза. Для этого выполнялась резекция корня аорты с оставлением устий коронарных артерий на площадках и створок клапана на комиссурах с участком стенки синусов 4-5 мм (рисунок 3, A, B). Дакроновый протез подшивался к основанию корня аорты швами на прокладках, швы накладывались ниже створок АК таким образом, что бы они проходили через фиброзный скелет основания выходного отдела левого желудочка (псевдо-кольцо). Комиссуры клапана подшивают внутрь дакроновой трубки таким образом, чтобы достичь коаптации створок. Операцию заканчивают подшиванием коронарных «пуговок» к неосинусам и наложением дистального анастомоза (рисунок 4). Особенностями операции реимплантации TD1 является цилиндрическая реконструкция, реимплантация устий коронарных артерий и максимальная стабилизация основания корня аорты (кольца). В первоначальной методике нет описания специфики синотубулярного соединения. Hvass сообщает об изменении данной техники таким образом, что дакроновый протез вшивается внутрь корня аорты к основанию створок клапана (в методе David – наоборот); такой метод реимплантации имеет недостатки – создается контакт створок АК с дакроновой тканью, и не создается стабильной фиксации фиброзного кольца клапана [35].



a *b* *c*

Рисунок 4 – Методика Tirone David 1, последовательно показаны этапы операции, впервые описанной в 1992 г.

Резекция аневризмы восходящей аорты, выкроены створки и столбики комиссур клапана с оставлением стенки синуса по краю 3-5 мм, для наложения швов. Устья коронарных артерий выкроены на «пуговках». Сосудистый (дакрон) протез пришивают к дистальной части восходящей аорты. Накладываются швы (6 стежков) ниже створок аортального клапана, которые проходят через фиброзную ткань в тоще вентрикул-оаортального перехода. Этими швами подшивается конец отобранного дакронового протеза. Выполнена фиксация протеза к основанию корня аорты. Стойки комиссур подвешиваются внутрь протеза непрерывным обвивным швом, подобно техники имплантации подкоронарного гомографта. Накладываютя анастамозы с коронарными артериями и дистальный межпротезный анастомоз.

В 1993 году Sarsam и Yacoub приводят серию наблюдений (10 больных) хирургического лечения аортальной недостаточности методом, названным «ремоделирование аортального кольца» [36].

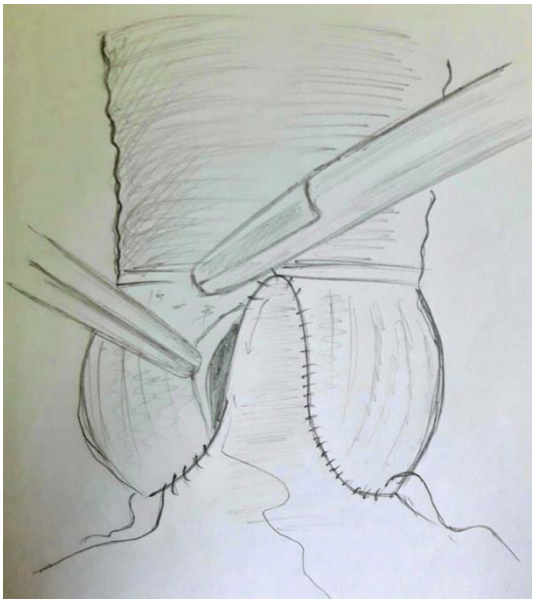


Рисунок 5 – Техника ремоделирования корня аорты Yacoub, 1993 год

Первая версия операции Якуба состояла из протезирования всех трех синусов Вальсальвы с реимплантацией устий коронарных артерий с использованием дакронового протеза выкроенного по типу трехлепестковой короны. Метод включает реимплантацию коронарных артерий, но не предусматривает стабилизации основания корня аорты и определения размеров синотубулярного соединения (рисунок 5). В своей методике авторы подчеркивают необходимость иссечения синусов аорты и подбора дакроновой трубки по диаметру равной диаметру основанию корня аорты. Разрезы на протезе выполняются для увеличения высоты фиксации комиссур клапана. Согласно названию, выполняется цилиндрическая реконструкция корня аорты, не создается специфического сужения в месте синотубулярного соединения, и нет стабилизации основания аорты. Преимуществами этого метода является техническая простота, чем при реимплантации, и позволяет более точно выполнить ресуспензию комиссуральных стоек. Последующие модификации операции Якуба включают в себя сужение в области синотубулярного гребня и создания выбухающих синусов.

Реимплантация или ремоделирование. В 1995 году David, Feindel и Boss сообщили о следующем шаге в эволюции методики Tirone David. В статье «Реконструкция аортального клапана при его недостаточности и аневризме корня аорты» [37], говорится о двух фундаментально разных методиках восстановления. Первой была техника реимплантации – TD1, которая применялась у больных с расширением синотубулярного сединения, разрушеными или дилятированными синусами Вальсальвы и при аннулоаортальной эктазии. Альтернативная методика, приведенная тут же, получила названия «Tirone Davide- 2» и описанная авторами как техника «ремоделирования», применялась у пациентов без аннулоаортальной эктазии, в большей части при деформации синусов Вальсальвы и необходимости коррекции синотубулярного соединения. В этой серии было описано 45 больных, смерть наблюдалась только в двух случаях. Девятнадцать больных были оперированы по методике реимплантации TD-1 и 26 – по методике ремоделирования TD-2, аортальный клапан был восстановлен путем реконструкции и протезирования всех синусов Вальсальвы устья коронарных артерий реимплантировались в протез по методике «пуговок». Не выполнялось никакого укрепления или уменьшения «аортального кольца», ремоделирования синотубулярного соединения также не было (т.е. уменьшения диаметра на 15% от диаметра выходного отдела ЛЖ).

Реимплантация корня аорты с реконструкцией псевдосинусов: методика сиэтлская техника. В 1995 году, Cochran и коллеги описали разновидность операции Tirone David, при которой применялась дакроновая трубка с формированными выпуклыми псевдосинусами, при той же технике сохранения клапана [38]. В сиэтлской технике аортальный клапан сохраняется и выполняется протезирование всех синусов Вальсальвы с реконструкцией неосинусов по специальной технике.

Выбухание этих неосинусов предотвращает контакт створки с дакроновой тканью. В нормальной аорте створки защищены динамической геометрией (расширение синусов, удлинение створок и корня аорты). Данная методика так же стабилизирует «кольцо аорты» с помощью проксимального шва расположенного ниже створок клапана, подобно тому, что и при методике TD-1, так же имеется дистальный шов, выше створок, предназначенный для максимальной фиксации стоек комиссур. Выкраивание лепестков на проксимальном конце протеза необходимо для формирование неосинусов.

Гибридная методика была предложена van Son и соавторами, при которой расширенный корень аорты уменьшается и реконструируется (коронарные артерии выкраиваются на пуговках) путем клиновидного иссечения стенок синусов Вальсальвы, и затем реимплантации восстановленного корня аорты внутрь дакроновой трубки, при внимательной реставрации высоты комиссуральных стоек; накладывается дистальный шов между протезом и корнем аорты, а затем подшиваются устья коронарных артерий [39]. Эта смешанная методика позволяет выполнять реконструкцию синусов, учитывая контроль диаметров синотубулярного соединения и фиброзного кольца, что и позволяет избежать контакта створки с дакроновым протезом, но при этом не происходит стабилизации основания корня аорты. Некоторые авторы оставляют «подушки» стенки аорты, теоретически для защиты створок от повреждения [40, 41].

В 1996 году, T. David описал еще одну разновидность своего метода ремоделирования (TD-2), который применяется при аннулоаортальной эктазии (методика TD-3). В данном случае аортальный клапан реконструируется путем протезирования всех тех синусов Вальсальвы, коронарные артерии реимплантируются в протез (рисунки 6, 7).

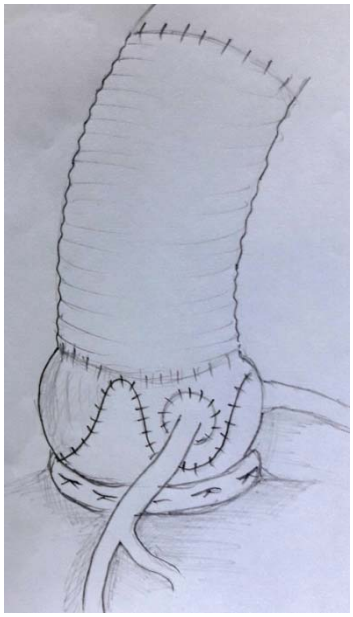


Рисунок 6 – Ремоделирование корня аорты
со стабилизацией фиброзного кольца клапана (TD-3)

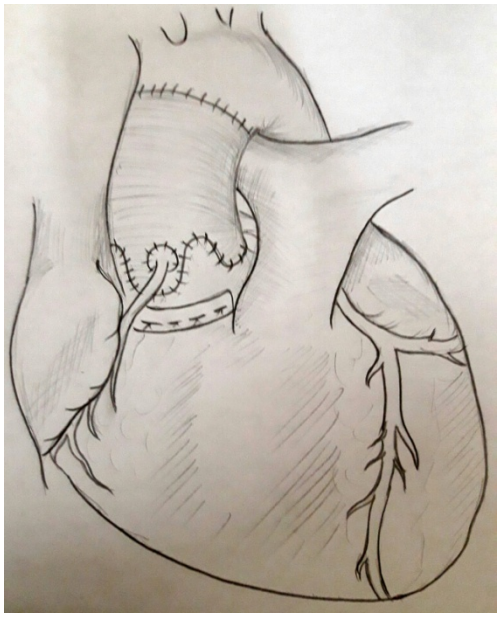


Рисунок 7 – Ремоделирование корня аорты
со стабилизацией фиброзного кольца клапана (TD-3)

Главным отличием данной техники является укрепление корня аорты и фиброзного кольца тефлоновой полоской. Эта методика также обеспечивает более легкую ресуспензию столбиков комиссур, подобно в технике Якуба, и ремоделирование синотубулярного соединения не зависит от размера выбранного протеза (рисунок 7 F-H). El Khoury и коллеги, сообщали о подобной методике, но они ограничивались только укреплением аортального кольца при его значительной дилятации; они также рекомендуют сохранять интактные синусы Вальсальвы [42].

Рисунки 6, 7. Ремоделирование Tirone David-3, 1996 г. Удаление пораженного корня аорты и восходящего отдела аорты. Аорта пересекается сразу выше синотубулярного гребня. Корень аорты мобилизирован до самого основания, коронарные артерии выкраиваются на площадках. Полоской тефлона выполяется укрепление фиброзной зоны выходного отдела левого желудочка, это дает

стабилизацию основания корня аорты, особенно необходимую при аннулоаортальной эктазии. Измерен диаметр аортального кольца (АА) из него вычтена толщина стенки аорты, для того что бы определить диаметр необходимого дакронового протеза. Методика ремоделирования корня аорты по TD-3: Замещение корня аорты выкроенным протезом. Адекватная ресуспензия комиссур. Реимплантация коронарных артерий в соответствующие синусы.

Восстановление синотубулярного гребня. Этот метод состоит из простого протезирования восходящей аорты с сопутствующей реконструкцией (т.е. сужением или редукционной аортопластикой) синотубулярного гребня для восстановления нормальной коаптации аортальных створок в корне аорты. Как отдельный метод его можно применять только при нормальных створках и синусах Вальсальвы, и когда фиброзное кольцо не нуждается в реконструкции. Не выполняется протезирования синусов, и реимплантации устий коронарных артерий. Эта операция не несет в себе укрепления и стабилизации основания корня аорты, по существу это метод восстановления синотубулярного соединения, который предложил Frater [31]. Доктор David включил этот подход в свою обновленную версию реимплантации (названную доктором Miller, как TD- 4), в которой он выбирает дакроновый протез на 4 мм больше необходимого и путем его циркулярной пликации создает синотубулярный гребень [43]. В технике реимплантации TD-5 или Miller-1 применяется дакроновый протез даже на 8 мм больше необходимого, за счет чего формируются синтетические псевдосинусы [43, 44]. Техника «Jena» является гибридной, в которой реконструкция аневризмы корня аорты выполняется путем пликации и иссечения порции синусов Вальсальвы (U-образно в коронарных и V-образно в некоронарном синусах), а синотубулярное соединение уменьшается за счет дакронового протеза (26-28 мм). Преимуществом этого метода является сохранение нативной ткани, которая контактирует со створками клапана, а также сохраняется динамические свойства корня аорты, но имеется риск дальнейшей дилятации зоны реконструкции [45].

Ремоделирование корня аорты с максимальной стабилизацией, сохранением створок и реконст рукцией синотубулярного соединения. Hopkins предложил свой метод реконструкции аортального клапана, но в сущности, это одна из разновидностей ремоделирования. В этой методике все синусы замещаются с реимплантацией устий коронарных артерий (подобно методам Yacoub или TD-2). Основание корня аорты укрепляется циркулярным швом, наложенным ниже створок (рисунок 8), это защищает расширение корня аорты в последующем, и поэтому применима к больным с врожденным дефектом соединительной ткани, таким как синдром Марфана. Как и в методиках Yacoub или TD- 3 выполняется несложная процедура ресуспензии комиссуральных стоек. В этой технике синотубулярное соединение ремоделируется до идеальных размеров, т.е. на 15% меньше диаметра аортального кольца, путем подборора дакронового протеза, меньшего по

Рисунок 8

диаметру, чем аортальное кольцо, синусы слегка выбухают наружу, что достигается более высоким вырезам на протезе, которые подвешиваются комиссуры клапана. Вырезы на протезе должны быть узкими, чтобы подчеркнуть место синтобулярного перехода, затем тефлоновой полоской производится сужение этого места. Путем сужения синотубулярного перехода, сохраняются функциональные потоки, описанные Да Винчи, которые предотвращают контакт створок клапана с дакроновым протезом. Эта реконструкция охватывает все компоненты корня аорты, сохраняя нативные створки аортального клапана.

Методика Hopkins-1. Суживающие полоски из тефлона накладываются циркулярно после иссечения патологически измененой аорты, и после мобилизации ее корня. Проксимальная полоска фиксируется серией матрасных швов, ниже расположения створок. Комиссуры сохранены с участком окружающей ткани 3-5 мм. Вырезы в проксимальном конце протеза сделаны несколько длиннее, что позволит подшить комиссуры еще выше, и за счет этого неосинусы будут выпуклыми. Наложен проксимальный шов в основании корня аорты, синусы замещены дакроновым протезом. Реимплантация коронарных устий, формирование сужения в месте, где подшиты комиссуры клапана.

Реимплантация корня аорты в протез Методика Florida Sleeve, описанной P. Hess в 2005 г. Восходящий отдел аорты пересекается на 1 см выше синотубулярного соединения, корень аорты выделяется по окружности до уровня аортожелудочкового контакта. Диаметр фиброзного кольца аортального клапана определяется с помощью измерителей стандартного размера. Также измеряется расстояние от основания корня аорты до устьев коронарных артерий и синотубулярного гребня по каждой комиссуре, что необходимо для подготовки протеза. Подбор протеза выполняется из расчета на 4–5 мм больше измеренного диаметра фиброзного кольца. Высота юбки протеза должна соответствовать измеренной высоте по каждой из комиссур, при этом все комиссуры должны находиться на уровне сино-тубулярного гребня протеза. Затем на протезе отмечаются расположения коронарных артерий, после чего в нем изготавливаются вертикальные прорези по обозначенным позициям в виде «замочной скважины». Длина щелей соответствует измеренному расстоянию от основания корня аорты до нижней части коронарных артерии. В месте расположения устьев коронарных артерий делают округлые отверстия. На следующем этапе накладывают субаннулярные П-образные швы плетеной нитью 3/0 на тефлоновых прокладках, располагая их горизонтально по кругу на 1–2 мм ниже створок аортального клапана изнутри наружу так, чтобы прокладки не касались створок (рисунки 9, 10). В месте между некоронарным и правым коронарным синусами швы необходимо располагать по контуру створок, чтобы избежать осложнений со стороны проводящей системы и мембранозной части межжелудочковой перегородки [46-48].

Случаи неудач реимплантации и ремоделирования аортального клапана. К неудачным клапансохраняющим операциям были отнесены методики, в которых нет адекватной стабилизации

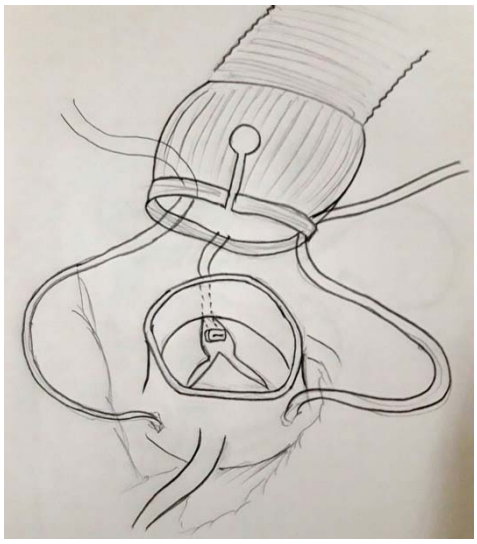


Рисунок 9 – Методика Florida Sleeve

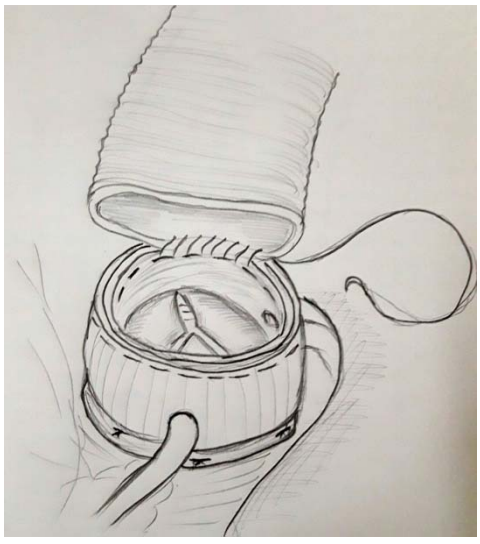


Рисунок 10 – Методика Florida Sleeve

основания аорты, и которые в отдаленном периоде приводят к дилятации аортального кольца [49]. Часть выходного тракта аорты формирует функциональное кольцо – фактически основания межлепестковых треугольников ниже места фиксации створок и является частью желудочковой гемодинамики [9, 10]. Сторонники техники реимплантации предполагают, что стабилизация кольца аорты, особенно при заболеваниях соединительной ткани, является важным моментом. Методы ремоделирования оставляют интактными эти треугольники, что должно положительно влиять на работу сохраненных створок и на длительность реконструкции, поскольку сохранены некоторые элементы динамического расширения корня аорты. Методы имплантации поддерживают или фиксируют эти межлепестковые треугольники.

Теоретической причиной ускоренной дегенерации створок является систолический контакт ее со стенкой протеза, вследствии отсутствия расширения синусов. Попытки неудачной реконструкции корня аорты при размере кольца 25- 27 мм были приписаны к травме створок из- за стеснения в протезе [11, 50-52]. Такие осложнения могут быть при реимплантации в цилиндрический протез или же во время ремоделирования, когда используется меньший размер протеза. Что бы избежать такого рода проблем разрабатываются протезы аорты с искусственными синусами Вальсальвы [53].

Pethig и коллеги, применив эхокардиографическое исследование, определили, что уровень, на котором происходит коаптация створок является важным фактором долгосрочности реконструкции. Они поделили всех больных на три группы в зависимости от уровня коаптации: А – коаптация створок на 2 мм выше плоскости кольца аорты; В – на уровне плоскости основания кольца; С – ниже уровня основания аортального кольца. Не было отмечено регургитации в группе А (n=56), в группе В выраженная АН имела место у 2-х больных (n=11); у всех больных из группы С имелась недостаточность АК (54).

Ранние методики ремоделирования корня аорты (Yacoub, David- 2) имели целью протезирование патологически измененных синусов Вальсальвы и сохранение динамических свойств аортовентрикулярной зоны. Однако, у больных с заболеваниями соединительной ткани могут развиваться отдаленные осложнения, связанные с дилятацией неукрепленных компоненто корня аорты, поэтому этот метод развивался в дальнейшем в сторону большей стабилизации основания аорты и синотубулярной зоны.

Доктор David сравнил свою технику реимплантации с развивающимися методами ремоделирования у больных с аневризмой корня аорты и обнаружил некоторое неравенство в долгосрочности этих методик. В то время как, 8-10 летняя выживаемость была превосходной, его исследование показало лучшие результы (т.е. свободу от развития умеренной и выраженной недостаточности АК) для техники реимплантации [49]. Он правильно отметил, что без укрепления, корень аорты может расширяться после многих из методик ремоделирования. В то время как, техника реимплантации теоретически имеет недостатки – контакт створок с синтетическими «синусами», в действительности это не является серьезной проблемой [49, 53, 55]. К тому же сегодня стали доступными и дакроновые протезы с выпуклыми синусами [53, 56-58].

Увеличение сроков службы восстановленных створок может достигаться хирургическим путем либо Сиэтлской техникой, либо применением протезов, предложенных Zehr [59], либо описанной методикой David [49], или же применением протеза синусов Вальсальвы в комбинации с трубкой меньшего диаметра, как это делается в клинике Mayo [50]. Однако, не совсем ясен механизм развития аортальной недостаточности в поздние сроки после операции ремоделирования, вероятнее всего из-за недостаточного укрепления остаточных фибромускулярных компонентов комплекса – корень аорты, что характерно для этой процедуры.

Кроме того, проблемы могут возникать из-за хрупкости и послеоперационной дегенерации и последующего утончения створок.

Выбор размера протеза для реимплантации или ремоделирования. Большое внимание уделялось выбору размеров протезов для различных методик реконструкции корня аорты. Методика реимплантации имеет преимущество – полная фиксация всего комплекса корня аорты и помещением клапана в дакроновый протез, однако его выбор отличается от такового при ремоделировании. Для реимплантации необходим выбор достаточно большого протеза, что бы не было увеличения площади коаптации створок. David подчеркнул, что синотубулярное соединение

может быть сформировано либо с помощью сужения протеза, либо путем подшивания протеза меньшего диаметра на уровне этого перехода если это необходимо; методика реимплантации вообще требует достаточно широкого протеза (30–34 мм), который идеально соответствует размеру не расширенного аортального кольца. Для выбора размера протеза в операциях ремоделирования описано несколько способов [3].

По методу M. Yacoub, для определения требуемой величины комиссуры натягиваются вертикально, определяется позиция створок и их способность к коаптации без пролапса; необходимый диаметр равен расстоянию между вершинами комиссур либо одной трети окружности аорты на уровне синотубулярного соединения. David предложил сначала нормализовать аортальное кольцо, а затем выбрать протез, учитывая что диаметр синотубулярного переходя меньше на 15% аортального кольца. Это соответствует морфометрии Kunzelman [2]. Группа Якуба предлагает так же измерить расстояния между комиссурами, при максимальной коаптации створок, и затем расчитать диаметр протеза. K.Morishita и соавт. Предложили использовать следующую формулу:

$$d=2/\sqrt{3} \times id,$$

где d – необходимый диаметр протеза, id – расстояние между вершинами комиссур.

Мнение T. David следующее: ориентировочный диаметр требуемого протеза равен длине свободного края створки аортального клапана минус 10% от полученной цифры, однако не существует абсолютно точной формулы, по которой можно рассчитать размер протеза, и решение этой задачи во многом зависит от опыта хирурга. При клапаносохраняющих операциях с реконструкцией синотубулярного гребня необходимый диаметр протеза равен удвоенной высоте створки аортального клапана. Доктор David отметил, что определение размеров корня аорты и синотубулярного соединения является больше искусством, нежели точной наукой [60]. В его руках выбор протеза для реимплатанции отличается от такового для ремоделирования [49]. Концепция выбора протеза при реимплантации основана на внешнем диаметре корня аорты (внутренний диаметр + толщина стенки), в то время как при ремоделировании – на внутреннем диаметре. При ремоделировании David основывается размерами створок и не рекомендует применения протезов меньше 30 мм во избежание ограничения синусов и последующего повреждения створок [49, 60, 66]. Для ремоделирования мы описали следующую методику. После иссечения синусов проводится истинного диаметра аортовентрикулярного соединения с помощью расширителя Гегара. Накладываются горизонтальные матрасные швы на вершину каждой комиссуральной стойки, которые затем потягиваются вверх до достижения соответствующего диаметра синотубулярного соединения, при этом проводят гидродинамический тест коаптации створок. Этот диаметр синотубулярной зоны обычно соответствует внутреннему диаметру выходному тракту аорты, который измерили Гегаром. Если площадь коаптации полулунных клапанов кажется недостаточной, то необходим подбор протеза меньшего по диаметру. Это очень простой метод (похож на артистический подход Девида), где комбинируется сужение протеза полоской из тефлона в области синотубулярного соединения и точный подбор высоты фиксации комиссур, что дает надежный результат реконструкции зоны синотубулярного соединения без деформации синусов.

Высота комиссуры между не коронарой – левая коронарной измеряется для определения размера трансплантата от линии, соединяющей надиры двух соседних створок (основание треугольника между рядами) до верхней части комиссуры. Это измерение соответствует размеру выбранного трансплантата; B. В трансплантате Gelweave Valsalva ™ (Vascutek Ltd, компания Terumo, Ренфрюшир, Шотландия) высота синусовой части равна ее диаметру, что соответствует маркированному размеру

Результаты реконструкции корня аорты с сохранением или восстановлением аортального клапана. Уровень операционной летальности колеблется от 0% до 6% [70,71] с выживаемостью за 7 лет 72-78 ± 8% [71,72]. Больные с аневризмой восходящей аорты имеют выживаемость ниже, чем больные с аневризмой корня аорты, приблизительно 36% выживают в 8-летний период [49]. Это низкий уровень выживаемости, вероятно связанный с пожилым возрастом больных с аневризмой ВА, а также сопутствующей сосудистой патологией. Реоперации после протезирования аортального клапана за 7- 8 лет имеют небольшую частоту и по данным многих авторов свобода от такого рода реопераций составляет 90-97% [68, 71, 73]. Умеренная аортальная недостаточность

является редкостью, особенно в течении первых 2-х лет после операции. Однако выраженная АН нередко приближается к 6%, хотя в некоторых сообщениях она достигает уровня до 37% [34, 55, 73-78]. Кроме хороших показателей выживаемости у больных, перенесших реконструкцию корня аорты, две трети наблюдаемых свободны от риска развития средней и выраженной степени недостаточности аортального клапана за 8 лет после операции (49). David отмечает превосходство техники реимплантации (над ремоделированием) с низким риском развития аортальной недостаточности в дальнейшем; средняя и выраженная АН имела места у 10% за 8-летний период, в то время как, при ремоделировании этот показатель составил – 45% [78]. Ганноверская группа добилась таких же успехов в реимплантации корня аорты, госпитальная летальность составила – 3,8 %, и 4% были реоперированны по поводу аортальной недостаточности [73].

Интраоперационная чрезпищеводная эхокардиографическая оценка является важной в определении долгосрочности выполненной операции [55]. Поздние осложнения (аортальная недостаточность) у больных с заболеваниями соединительной ткани выявляются чаще, при методиках, где нет максимальной стабилизации основания корня аорты («кольца») [60, 66-69, 78-80].

У больных с аневризмой корня аорты и непроврежденным комплексом корня, у которых реконструкция состояла из нормализации диаметра синотубулярной зоны непосредственные и промежуточные функциональные результаты весьма хороши, и более двух третей больных свободны от развития АН в сроки 8-10 лет после операции; однако, общая выживаемость этих больных относительно остается низкой, и только треть остается живыми к 8 году, возможно играют роль и сопутствующие заболевания сосудов и возраст пациентов [49].

Для сравнения техник реконструкции доктор Gott с коллегами, рассмотрели результаты протезирования у 235 больных с синдромом Марфана, из которых 232 перенесли операцию Бентала и протезирование корня аорты. В этой группе не было отмечено смертей за 30 дней, 85% этих больных были живы ко времени публикации этой статьи, а свобода от реоперации за 20 лет составила 74% [81]. В японском исследовании, использовался гофрированный кондуит, оперативная летальность составила 8,3%, а акттуарная выживаемость к 5 году была на уровне 82,7 ± 4,8% [82].

Edwards с коллегами, используя базу данных National STS Cardiac Surgery определили нормы оперативной летальности для изолированного протезирования аортального клапана- 4% ; для больных с плановым протезированием АК- 3,3% [83]. Эти данные говорят о том, что у выборочных больных, плановые клапансохраняющие операции в настоящее время выполняются в большинстве центров с уровнем смертности приближающимся или лучшим, чем для изолированного протезирования аортального клапана.

Заключение. Прогресс этих методик произошел после понимания функциональной анатомии комплекса корень аорты. Предварительные результаты таких операций поддерживают интерес к их применению, но идеальная и безопасная техника реконструкции, особенно при заболеваниях соединительной ткани, например синдром Марфана, должна определиться после более долгого промежутка послеоперационного наблюдения (43). Сегодня имеется много методик реконструкции корня аорты, некоторые первоначальные техники были заменены или же модифицированы этими же авторами. Хирург должен рассматривать корень аорты как комплекс элементов, и стремиться к оптимизации его функциональной анатомии у каждого больного индивидуально. Разработка новых видов протезов так же облегчают эту задачу. Знание специфической анатомии и истории заболевания каждого пациента должно помочь в успешной реконструкции комплекса корень аорты с продолжительным эффектом и низкой летальностью.

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ҚОЛҚА ҚАҚПАҚШАСЫНЫҢ ЖЕТІСПЕУШІЛІГІМЕН ҚОСАРЛАНҒАН ҚОЛҚА ТҮБІРІНІҢ АНЕВРИЗМАСЫ КЕЗІНДЕГІ РЕКОНСТРУКТИВТІ ОПЕРАЦИЯЛАР

Аннотация. Қазіргі танда қолқа түбірінің аневризмасы кезіндегі реконструктивті операцияларға кардиохирургия саласында қызу талқылануда. Соңғы отыз жылдықта қолқа түбірінің аневризмасы кезінде жасалатын операциялардың бірнеше әдіс-тәсілдері ұсынылған. Олар бір-бірінен жасалу техникасы және түзету жүргізілетін анатомиялық аймағына байланысты ерекшеленеді. Бұл әдіс-тәсілдердің мақсаты қызметі сақталған жармаларды сақтап, қолқа түбірінің басқа бөліктерінің тұқтандыру болып табылады. Бұл шаралардың беріктігі мен нәтижесінің ұзақ сақталуының маңыздылығы антикоагулянтарды қолдануда қажеттіліктің болмауында жатыр. Хирургиялық түзетудің әдіс-тәсілдерін таңдауда осы уақытқа дейін пікірталас бар, және әр жағдайда таңдау хирургқа қалады. Бұл шолуда қолқа түбірінің реконструкцивті операцияларын таңдауды жүйелі түрде қаралған және операциялардың графикалық иллюстрациялармен берілген. Операциялардың иллюстрациялармен суреттелуі хирургтың әдіс-тәсілдерді таңдауын жеңілдетеді.

Түйін сөздер: аорта түбірінің аневризмасы, қолқа қақпақшасының жеткіліксіздігі, аорта түбірінің реконструкциясы.

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miRNA: ACHIEVEMENTS, MISCONCEPTIONS, PERSPECTIVES

Abstract. Among small RNAs miRNAs play an important role, they carry out the regulation of gene expression at the post-transcriptional level. The paper discusses the properties of miRNAs and their interaction with mRNAs. It is shown the role of miRNA in the regulation of the expression of protein-coding genes involved in various metabolic processes and the development of cardiovascular, oncological and neurodegenerative diseases. The features of miRNA binding sites in 5'UTR, CDS and 3'UTR of mRNA of target genes have been established. There were shown the advantages of the MirTarget program prior to known search programs for miRNA binding sites with mRNA. In mRNA of many candidate genes of various diseases, single miRNA binding sites and miRNA binding site clusters are detected in 5'UTR, CDS and 3'UTR of mRNA. There was found miRNA binding sites that encode oligopeptides in proteins in mRNA of transcription factor genes. It was analyzed the interaction of miRNA with mRNA of candidate genes involved in cardiovascular, oncological and neurodegenerative diseases. The properties of unique miRNAs binding sites in mRNAs of several hundred genes were discussed. There were considered the features of the interaction of mRNA with miRNA in the RISC complex. Discussed the role of miRNA in the regulation of gene and genome expression through the interaction of genes involving miRNA host genes. It is proposed the hypothesis of regulation of gene and genome expression involving miRNA. Shown the role of miRNA as an integrating system for the mutual regulation of gene expression in the cell and in the body.

Keywords: miRNA, mRNA, genes, binding sites, bioinformatic programs.

Introduction. During the period of studying miRNA, many original articles and reviews have been published and important properties of the functioning of these molecules have been established and discussed [1-7]. The conducted researches have allowed to identify the features of miRNA properties and their interaction with mRNA. The obtained knowledge was the basis for the use of synthetic molecules of siRNA by which it is possible to completely suppress the translation process or destroy mRNA. For the development of this method of turning off genes, a group of scientists was awarded the Nobel Prize. However, despite significant success in studying the interaction of miRNA with mRNA, with rare exceptions, miRNA cannot be used for practical purposes, in particular, in diagnosis and therapy of diseases. The reasons for the poor performance of miRNA studies are inadequate assumptions about their properties that brake on the identification of the biological role of miRNA and the use of miRNA in biology, biotechnology, medicine, etc.

Small noncoding RNAs (ncRNAs) include transfer RNAs (tRNAs), antisense RNAs (asRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), micro RNAs (miRNAs), Piwi-interacting RNAs (piRNAs), competing endogenous RNAs (ceRNAs), tRNA-derived small RNAs (tsRNA) and small interfering RNAs (siRNAs), which play an important role in the expression of genes and genomes [8, 9]. The miRNAs participate in the regulation of gene expression at the post-transcriptional level, suppressing the translation process. The miRNAs are nanoscale molecules with a length of five to nine nanometers consist of 18 to 27 nucleotides. Consequently, the name of the microRNA contradicts the nanoscale of these molecules. The name of the microRNA has another drawback - it does not reflect the function of these molecules. However, the used “miRNA” abbreviation can be treated as mRNA-inhibitory RNA (miRNA), which corresponds to the function of these molecules. The term “microRNA” should be phased out as an inadequate term. The interaction of mRNA with miRNA is studied using different approaches: how much miRNA binds to one gene; how many genes are targeted by a single miRNA; what are the criteria for predicting sites and the energy of interaction of mRNA and miRNA; whether there are gene any preferences for miRNA binding; what proteins functions encoded by target genes of specific miRNA are; what are functional links of miRNA in the implementation of post-transcriptional regulation of gene expression; how, what and to what extent the synthesis of miRNA is regulated and etc.

The present work is devoted to the consideration of miRNAs properties and their interaction with mRNAs, which in our opinion can substantially clarify the existing problems in the study and application of miRNAs for practical purposes.

Materials and methods. The nucleotide sequences of mRNAs of genes were downloaded from NCBI GenBank (http://www.ncbi.nlm.nih.gov). Nucleotide sequences of miRNAs were downloaded from the miRBase database (http://mirbase.org) and borrowed from the article of Londina E. et al. [10]. miRNA binding sites in 5'-untranslated regions (5'UTRs), coding domain sequences (CDSs) and 3'-untranslated regions (3'UTRs) of several genes were predicted using the MirTarget program [11]. This program defines the following features of binding: a) the origin of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in 5'UTRs, CDSs and 3'UTRs of the mRNA; c) the free energy of hybridization (ΔG, kJ/mole); and d) the schemes of nucleotide interactions between the miRNA and the mRNA. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of miRNA binding with its perfect complementary nucleotide sequence). The miRNA binding sites located on mRNAs had ΔG/ΔGm ratios of 90% or more. The program identifies the positions of binding sites on mRNA, beginning from the first nucleotide of the mRNA's 5'UTR. The MirTarget program found hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C. The distances between A and C were equal to those between G and C, A and U, and G and U. The numbers of hydrogen bonds in the G-C, A-U, G-U and A-C interactions were found to be 3, 2, 1 and 1, respectively. The free binding energies of these nucleotide pairs were taken as the same ratio, i.e., 3, 2, 1, and 1, respectively.

Results and discussions. After detecting miRNA and establishing their interaction with mRNA, it became necessary to develop programs for predicting miRNA binding sites in mRNA. This need derives from the fact that more than 6000 miRNA encoded in the human genome can potentially bind to all mRNA of 20000 genes and their isoforms, encoded in the human genome. It has been established that miRNA can bind to mRNA blocking the translation [12]. Therefore, it needs to create programs that establish miRNA binding sites in mRNA and quantitative characteristics of the interaction of these molecules, evaluating the effectiveness of this binding.

The need to take into account the interaction of miRNA with mRNA throughout the entire miRNA nucleotide sequence, rather than the "seed", is due to several factors. For the high specificity of the interaction of miRNA with mRNA, the entire length of miRNA must be taken into account. It is same as applying primers, of at least 20 nucleotides length in polymerase chain reaction, when it is necessary for amplification to specifically choose only one nucleotide sequence among numerous nucleotide sequences. Another reason for using the full miRNA nucleotide sequence is that during the evolution process, a part of miRNA other than "seed" will vary, if it is not a critical site in interactions of miRNA with mRNA. Confirmation of the need to maintain the entire length of miRNA is the high conservatism of miRNA in organisms that have diverged over tens of millions of years of species evolution. According to miRBase,

the nucleotide sequences were identical in miR-200c-5p of human, mouse, rat, miR-216a of human, bull, mouse, miR-574-5p and miR-574-3p of human, mouse, rat, pig, etc.

TargetScan program finds the binding site for miR-3180*,* miR-3180-3p, miR-3196, miR-6816-5p in mRNA of *TGFB1*, gene coinciding with MirTarget program (table 1). However, with equal probability TargetScan indicates other sites that have an identical "seed" region. Such prediction increases the number of false-positive sites by tens of times, which makes prediction of sites extremely ineffective. Similarly, other programs [13] based on the search for miRNA binding sites in mRNA over the homology of 6-8 nucleotides "seed" at the 5' end of miRNA are also inadequate. The MirTarget program predicts the most likely binding sites for miR-6816-5p in CDS of *TGFB1* mRNA. Other miRNAs have less free binding energy and a smaller value of ΔG/ΔGm, which indicate a weak interaction with mRNA of *TGFB1*. Thus, miRNA-mRNA interaction schemes presented in table 1 clearly show the inadequacy of the TargetScan program and other programs based on the use of "seed" [13].

Table 1 – Schemes of miRNA interaction in mRNA of *TGFB1* gene

Program MirTarget	Program TargetScan

TGFB1, miR-6816-5p, 2051, CDS,-113, 90, 21
5'-GCUGAGGUCCCGCCCCGCCCCG-3'
  || | || |||||||||||||
3'-CGUC-CCUGGACGGGGCGGGGU-5'
	TGFB1, miR-6816-5p, 3'UTR, 21
	5'-...NNGGUCCCGCC**CCGCCCC**G-3'
	  |||||||
	3'- CGUCCCUGGACGG**GGCGGGG**U-5'

TGFB1, miR-3196, 2054, CDS, -98, 88, 18
5'-GAGGUCCCGCCCCGCCCCG-3'
  |||||||| | ||||||||
3'-CUCCGGGG-ACGGCGGGGC-5'
	TGFB1, miR-3196, 3'UTR, 18
	5'-..NNGGUCCCGCCCCGCC**CCGCCCC**G-3'
	  ||||| |||||||
	3'- CUCCGGGGAC-**GGCGGGG**C-5'

TGFB1, miR-3180-3p, 471, 5'UTR, -104, 80, 22
5'-AGCCCUCGGGAGUCGCCGACCCG-3'
  |||||||||||| | ||| ||||
3'-CCGGAGGCCUUC-GAGGCGGGGU-5'
	TGFB1, miR-3180-3p, 3'UTR, 22
	5'-..NNGGUCCCGCCCCGCC**CCGCCCC**G-3'
	  |||||||
	3'- CCGGAGGCCUUCGA**GGCGGGG**U-5'

TGFB1, miR-3180-5p, 227, 5'UTR, -106, 74, 25
5'-CCACUGCGGGGAGGAGGGGGAGGAGG-3'
  | || ||||| |||| | |||||
3'-GCUG-CACCCCGCCUCGCAGACCUUC-5'
	TGFB1, miR-3180 *,* 3'UTR, 19
	5'-..NNGGUCCCGCCCCGCC**CCGCCCC**G-3'
	  |||||||
	3'- GAGGCCUUCGA**GGCGGGG**U-5'

Note: Gene; miRNA; the beginning of binding site; the miRNA region; the free energy change (ΔG, kJ/mole); the ΔG/ΔGm (%); length of miRNA (nt). In bold type highlighted the “seed” nucleotides.	

One of the first misconceptions in the study of miRNA was the assumption that miRNA binds only (or predominantly) in the 3'UTR mRNA of human genes [14]. However, miRNAs do not have the property of distinguishing binding sites in 5'UTR, CDS and 3'UTR. miRNA interact with mRNA on the basis of physicochemical properties of these molecules. Therefore, the interaction site can be located in any mRNA region and as yet unknown prohibitions on the location of such sites throughout the mRNA nucleotide sequence. The conditions for the successful interaction of miRNA with mRNA are energy characteristics and conformational properties of this interaction. The proposed assumption of preferential miRNA binding in the 3'UTR contradicts several established properties of miRNA and mRNA. The proposed assumption of preferential miRNA binding in the 3'UTR contradicts several established properties of miRNA and mRNA. Considering that known miRNAs (miRBase) have differences in GC-content in the same range as in human genes, it was logical to assume that the probability of binding miRNA in 5'UTR, CDS and 3'UTR of mRNA will correlate with the GC-content of genes and corresponding miRNAs.

In numerous publications, sites of miRNA interaction with mRNA were studied only in the 3'UTR. This is because practically all programs for detecting miRNA binding sites are predicted only in this mRNA region. Several publications show the interaction of miRNA with mRNA in 5'UTR and CDS [15-19].

Using the MirTarget program, we have found binding sites in 5'UTR, CDS and 3'UTR of many animal and plant genes. It is shown, that miRNAs on average have a large free energy of interaction in the 5'UTR, because GC-content of miRNA and mRNA binding sites have greater importance, than in the

CDS. In turn, on the average free energy of miRNA interaction in the CDS is larger than miRNA interaction in the 3'UTR mRNA. The share of binding sites in 5'UTR, CDS and 3'UTR mRNA sites accounts for 20%, 54% and 26%, respectively. Based on 1000 nucleotides of 5'UTR, CDS, and 3'UTR length, the binding site density in these sites is 24.2, 4.3 and 5.1 sites on 1000 nucleotides, respectively. This distribution of binding sites in 5'UTR, CDS and 3'UTR has biological significance. Since binding of miRNA to mRNA can lead to stop translation and to separation of abortive polypeptides, then for energy saving is beneficial to stop translation at beginning of this process by binding of miRNA in the 5'UTR. For the same reason, miRNA binding sites in the CDS are generally located at beginning of CDS.

Another property of location of miRNA binding sites in 5'UTR, CDS, and 3'UTR is the optimization of their localization in sites with overlapping of binding sites of different miRNAs. This allows several and even dozens of miRNAs to interact with mRNA in a short area. This feature is particularly important when localizing of miRNA binding sites in the CDS, as these sites may encode oligopeptides is not involved in the functioning of protein.

Polysites increase the probability of binding miRNA with mRNA. Typically, among many sites there is a site that interacts with miRNA more effectively than other sites. Polysites of miRNA binding sites encode polyaminoacids that have functional significance, for example, interact directly with DNA, or with a protein bound to DNA [20].

miRNAs are inhibitors of translation reaction, and a biochemical approach to evaluating the action of inhibitors is applicable to them. Below it is shown a diagram of interaction of mRNA with miRNA included in RISC:

$$\text{miRNA} + \text{RISC} \leftrightarrow \text{miRISC} + \text{mRNA} \leftrightarrow \text{miRISC} \equiv \text{mRNA} \rightarrow \text{RISC} + \text{res-mRNA},$$

where miRISC is the association of all proteins of the RISC complex with miRNA; miRISC ≡ mRNA is miRISC complex with mRNA due to hydrogen bonds; res-mRNA is restricted mRNA. The diagram shows the following processes. The miRNA binds to a group of RISC proteins, forming miRISC. Then, miRISC binds to mRNA via hydrogen bonds and blocks protein synthesis, or miRISC cuts mRNA, which is further destroyed by cytoplasmic restriction enzymes. Binding stage of miRISC with mRNA is reversible and in the absence of their interaction, mRNA can again serve as a template for protein synthesis. It follows from this scheme that different effects can be observed on the ratio of concentrations of miRNA and mRNA. Assume that miRNA is complementary to the binding site in mRNA, that is, it has a high affinity for mRNA. Despite this, at low concentrations of miRNA compared to mRNA, the complex will have little effect on protein synthesis, since it will block the small quantity of mRNA. If concentration of miRNA is comparable or greater than the concentration of mRNA, protein synthesis will be slowed down or completely inhibited. With an average affinity of interaction of miRNA with mRNA, effect of complete inhibition of protein synthesis can be achieved with miRNA concentrations much greater than mRNA. Therefore, when calculating the probability of the degree of inhibition of gene expression by miRNA, it is not sufficient to know the affinity of miRNA for mRNA and the ratio of their concentrations.

In addition, it is necessary to take into account degree of intramolecular interaction of miRNA binding sites with other sites of mRNA. As a rule, intramolecular interactions are weaker than those of miRNA with mRNA, but cases of almost or completely complementary intramolecular interaction of these sites are known. In this case, energy is needed to break bindings of miRNA with mRNA comparable to binding energy of miRNA to mRNA. Therefore, calculation of probability of binding miRNA to mRNA only on basis of known programs for prediction of binding sites is not adequate, since it does not take into account intramolecular interactions in mRNA.

Considered variants of conditions for interaction of miRNA with mRNA are realized in cells. It is known that the concentration of miRNA can vary hundreds of times in cells [21]. Synthesis of mRNA, depending on the functional state of cell, can also vary hundreds of times [22]. In addition, gene expression and miRNA synthesis are tissue-specific [23]. Even in experiments of study of miRNA effect on protein synthesis concentrations of miRNA and mRNA are often not indicated. An important factor in studying of miRNA interaction with mRNA under *in vivo* is difficult-to-take effect of intronic miRNA (in-miRNA), which, as a rule, is synthesized coherently with the expression of host gene. Of all human

miRNA intergenic miRNA (ig-miRNA) constitute 40%, intronic - 52%, exonic - 5%, and the rest of 3'UTR and 5'UTR.

Study of ig-miRNA and in-miRNA in a comparative sense is necessary because ig-miRNA precursors are transcribed from DNA with the help of their promoters, and in-miRNAs mainly ripen from pre-mRNA and only a part of their precursors are transcribed directly from DNA. In addition, expression of in-miRNA depends on expression of host genes in introns in which they are located, whereas ig-miRNA is expressed independently.

Prospects of miRNA application in directed regulation of metabolism and diagnosis of diseases and therapy. Possibilities of using of miRNAs for these purposes of medicine are enormous, because miRNAs are endogenous, physiological regulators of biological processes, they escape from the action of the protective mechanisms of body (immune system, proteases, nucleases, etc.). Diagnostic method using miRNA can be based on miRNA associations and their target genes that interact almost complementarily. Confirmation of stability of such association is the preservation of orthologous associations in animals of related species. We have shown that such associations are conservative for tens of millions of years after the divergence of species.

Table 2 – Schemes of interaction of miRNA with mRNA of candidate genes subtypes of breast cancer

Triple-negative (Basal) subtype	
ATM, miR-619-5p, 9793, 3'UTR, -119, 98 5' - GGCUCACGCCUGUAAUCCCAGC - 3' 3' - CCGAGUACGGACAUUAGGGUCG - 5'	*AXL*, miR-1273g-3p, 3'UTR, -115, 98 5' - CCCAGGCUGGAGUGCAGUGGU - 3' 3' - GAGUCCGACCUCACGUCACCA - 5'
IAPP, miR-5096, 876, 3'UTR, -113, 100 5' - GCCUGACCAACAUGGUGAAAC - 3' 3' - CGGACUGGUUGUACCACUUUG - 5'	*CEACAM5*, miR-5095, 3229, 3'UTR, -115, 98 5' - CGCGGUGGCUCACGCCUGUAA - 3' 3' - GCGCCACCAAGUGCGGACAUU - 5'
ERBB3, miR-619-5p, 5104, 3'UTR, -121, 100 5' - GGCUCAUGCCUGUAAUCCCAGC - 3' 3' - CCGAGUACGGACAUUAGGGUCG - 5'	*IL11*, miR-1273f, 1466, 3'UTR, -102, 98 5' - CACUGCAACCUCCACCUCC - 3' 3' - GUGACGUUGGAGGUAGAGG - 5'
MAGEA10, miR-1273e, 2188, 3'UTR, -110,95 5' - UCCGCCUCCUGGGUUCAAGCGA - 3' 3' - AGGUGAAGGACCCAAGUUCGUU - 5'	*MAGEA10*, miR-1273e, 2188, 3'UTR, -110, 95 5' - UCCGCCUCCUGGGUUCAAGCGA - 3' 3' - AGGUGAAGGACCCAAGUUCGUU - 5'
Her2 subtype	
GTF2E1,miR-1273g-3p, 1720, 3'UTR,-108,93 5' - CCCAGGCUGGAGUGCAAUGGC - 3' 3' - GAGUCCGACCUCACGUCACCA - 5'	*MAZ*, miR-3960, CDS, -118, 93 5' - CCCCCGCCUCCGCCGCCACU - 3' 3' - GGGGGCGGAGGCGGCGGCGG - 5'
ADAM17, miR-619-5p, 3466, 3'UTR,-121,100 5' - CCCAGGCUGGAGUGCAGUGGU - 3' 3' - GAGUCCGACCUCACGUCACCA - 5'	*ERBB3*, miR-619-5p, 5104, 3'UTR, -121, 100 5' - GGCUCAUGCCUGUAAUCCCAGC - 3' 3' - CCGAGUACGGACAUUAGGGUCG - 5'
Luminal A,B subtype	
HMGA2, miR-3960, 512, 5'UTR, -108, 86 5' - CCUCCACCUCCACCGCCACC - 3' 3' - GGGGGCGGAGGCGGCGGCGG - 5'	*MAPT*, miR-6756-3p, 3'UTR, -98, 85 5' - CUGGGCAGAGGGGAGAGGAA - 3' 3' - GACCCGUCCCUCCUUCCCCU - 5'
MCM7, miR-4433b-5p, 248, 5'UTR, -100, 85 5' - GCGGGAGCGGGGGUGGGGUGC - 3' 3' - UGUCCUCACCCCCACCCUGUA - 5'	*MCM7,* miR-670-3p, 2769, CDS, -89, 86 5' - CUCUGGAUGAAUAUGAGGAGC - 3' 3' - AGGACUUACUUAUACUCCUUU - 5'
Notes. Gene; miRNA; the beginning of binding site; the miRNA region; the free energy change (ΔG, kJ/mole); the ΔG/ΔGm (%); length of miRNA (nt).	

Table 2 shows the interaction characteristics of some miRNAs with mRNAs of genes. A high degree of homology of nucleotide sequences of miRNAs in binding sites of mRNAs of different candidate breast cancer genes is seen from the schemes. This can serve as an example of associations for use in the diagnosis of breast cancer.

We discovered a new property of miRNA-mRNA interaction: several miRNA binds in a short region of mRNA sequence that contains binding sites for these miRNAs.

Using MirTarget program, it was found that ten miRNAs can bind in the 5'UTR of mRNA in a region from 110 nucleotides (nt) to 148 nt (table 3). The beginning of miR-9-25082-3p and miR-1-1819-3p binding sites coincides and locates from 110 nt. The free energy of interaction of these miRNAs is equal to -121 kJ/mole and -123 kJ/mole, with ΔG/ΔGm equal to 85% and 86%, respectively. From 112 nt, miR-9-20317-3p and miR-X-48174-3p binding sites started, which interacted with mRNA with a value of free energy of interaction -129 kJ/mole and -121 kJ/mole, with ΔG/ΔGm equal to 87% and 85%, respectively. miR-17-39416-3p binding site starts from 113 nt and in this miRNA 92% of nucleotides are complementary to mRNA, with a free interaction energy of -121 kJ/mole. Binding sites for next pair of miR-5-15733-3p and miR-7-20203-3p are located started from 115 nt. The free energy of interaction of these miRNAs is -127 kJ/mole and -121 kJ/mole, with the value of ΔG/ΔGm equal to 86% and 90%, respectively. The miR-9-27797-5p has two binding sites in the 5'UTR of mRNA at 118 nt and 124 nt positions. The free energy of interaction of this miRNA is -121 kJ/mole and -127 kJ/mole, with ΔG/ΔGm equal to 85% and 90%. The presence of two miR-9-27797-5p binding sites provides for it an increased probability of interaction with mRNA of *MMP2* gene. miR-12-17092-3p and miR-9-24743-3p binding sites are located from 124 nt and from 125 nt. ΔG/ΔGm value is 89%, and a free energy of miR-12-17092-3p and miR-9-24743-3p interaction with mRNA is -123 kJ/mole and -127 kJ/mole.

Table 3 – Characteristics of miRNAs interaction in the 5'UTR mRNA of *MMP2* gene

miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
miR-9-25082-3p	110	-121	85	24
miR-1-1819-3p	110	-123	89	23
miR-9-20317-3p	112	-129	87	24
miR-X-48174-3p	112	-121	85	24
miR-17-39416-3p	113	-121	92	22
miR-5-15733-3p	115	-127	86	24
miR-7-20203-3p	115	-121	90	22
miR-9-27797-5p	118	-121	85	24
miR-9-27797-5p	124	-127	90	24
miR-12-17092-3p	124	-123	89	22
miR-9-24743-3p	125	-127	89	23

At the 5'UTR length of 312 nt, binding sites of ten miRNAs are located compactly in a region of 38 nt. Such a compact arrangement of binding sites of several miRNAs facilitates their preservation in the process of evolution. Overlap of miRNA binding sites nucleotide sequences suggests their competition at inhibition of mRNA translation, since one miRNA in the RISC complex interferes with interaction of remaining miRNAs with this site. As a result, the control of mRNA translation is reliably ensured by several miRNAs, which seems to be necessary to suppress the increased synthesis of MM2 proteinase. It should be noted that the location of translation inhibitory miRNA binding sites in the 5'UTR allows cell to save energy on abortive proteins synthesis comparing with miRNA binding occurring in protein coding region or in the 3'UTR with protein synthesis interrupting in these regions.

Binding sites for many miRNAs have been identified in mRNA of *ZFHX3* gene (table 4). Binding sites of miR-15-36707-5p and miR-5-15548-3p are located in the 5'UTR with arranged location of nucleotide sequences and if these miRNAs are present in the cell simultaneously, they will compete for the binding site. The effect of each of the miRNAs will depend on the ratio of their concentrations, and

Table 4 – Characteristics of miRNAs binding sites in the 5'UTR mRNA of *ZFHX3* gene

miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
miR-15-36707-5p	26	-125	88	23
miR-5-15548-3p	31	-123	88	23
miR-19-30988-5p	187	-125	87	23
miR-16-36024-3p	189	-123	87	23
miR-17-39126-5p	195	-123	97	21
miR-4-11437-3p	267	-123	88	23
miR-9-20317-3p	278	-132	89	24
miR-1-1819-3p	285	-125	91	23
miR-2-5973-3p	297	-123	89	24

overall the expression of the *ZFHX3* gene will be determined by the total concentration of miR-15-36707-5p and miR-5-15548-3p, since they have close free interaction energies (ΔG are equal to -125 kJ/mole and -123 kJ/mole, respectively) with mRNA of *ZFHX3* gene.

Binding sites of miR-19-30988-5p, miR-16-36024-3p and miR-17-39126-5p also form a clusters with arranged location of nucleotide sequences (table 4). Binding sites of miR-4-11437-3p, miR-9-20317-3p, miR-1-1819-3p and miR-2-5973-3p form another cluster of multiple binding sites. For these two multi-sites, the same reasoning applies as for miR-15-36707-5p and miR-5-15548-3p which binding sites located in front of them. In general, the expression of *ZFHX3* gene will depend on nine miRNAs that bind in the 5'UTR.

A cluster of miRNA binding sites was revealed in the CDS of mRNA of *ALK* gene (table 5). The miRNA binding sites from 3,387 nt to 3,424 nt are formed a cluster. In the site with length of 37 nt there are binding sites for nine miRNAs: miR-1281, miR-11-29785, miR-13-35476-3p, miR-17-39011-3p, miR-7-20459-3p, miR-9-25099-3p, miR-6792-3p, miR-1-2802-3p, miR-22-40302-3p, miR-X-48174-3p. There are 4-5 binding sites for miR-1281 from 3389 nt to 3421 nt, five binding sites for miR-9-25099-3p from 3387 nt to 3421 nt, three binding sites for miR-11-29785 from 3391 nt to 3425 nt, two binding sites for miR-13-35476-3p from 3394 nt to 3420 nt, three binding sites for miR-7-20459-3p from 3395 nt to 3424 nt. The oligopeptide EWAGGGGGGGGA is conserved in the human ALK protein and 54 animal species, including rat, mouse and rabbit. The mRNA nucleotide sequences adjacent to the binding sites of nine studied miRNAs are variable, which are reflected in the variability of amino acids of flanking oligopeptide EWAGGGGGGGGA.

Table 5 – Characteristics of miRNA interaction with CDS mRNA of *ALK* gene associated with the development of NSCLC

miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
miR-9-25099-3p	3387 ÷ 3399 (5)	-104 ÷ -108	82 ÷ 85	22
miR-17-39011-3p	3388 ÷ 3394 (2)	-110 ÷ -113	84 ÷ 85	23
miR-11-29785	3391 ÷ 3404 (3)	-102 ÷ -106	86 ÷ 89	21
miR-6792-3p	3391	-110	90	22
miR-1-2802-3p	3395	-113	90	22
miR-13-35476-3p	3394 ÷ 3398 (2)	-110	85	22
miR-22-40302-3p	3395	-117	89	22
miR-7-20459-3p	3395 ÷ 3404 (3)	-98 ÷ -102	82 ÷ 86	20
miR-X-48174-3p	3394	-125	88	24

Cluster of miRNA binding sites were identified in the 3'UTR mRNA of *FOXP1* gene (table 6). Binding sites of five miRNAs were located at a region 30 nt in length. The total length of five binding sites is 113 nt, which is almost four times the length of the cluster.

The organization of binding sites in clusters allows gene to significantly reduce its length and preserve dependence on the influence of many miRNAs (tables 3-6).

Table 6 – Characteristics of miRNAs interaction in the 3'UTR mRNA of *FOXP1* gene

miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
miR-10-29282-3p	5952	-104	89	23
miR-10-29282-3p	5970	-104	89	23
miR-19-42814-5p	5953	-106	91	23
miR-19-42814-5p	5955	-104	89	23
miR-6-17605-3p	5960	-110	93	21

in-miRNAs, which co-expressed with the host gene, can be considered as agents realizing the interaction between genes.

In our opinion, the main mechanism of signal transfer from regulator gene to target gene is through miRNA. These molecules are co-expressed together with the host gene (regulator gene) and directly effect on target gene expression at the translation level. Thus, rapid signal transmission within the cell, between cells and tissues is achieved, since miRNAs are much faster than proteins leaving the cell and circulating in the body interacting with virtually all tissues. Thus, miRNAs can serve as integral regulators of the expression of genes and genomes, depending on their physicochemical properties and host genes. There are some miRNAs, which can effectively regulate the expression of hundreds of genes (unique miRNAs). Probably, therefore, the targets of such miRNAs are predominantly transcription factor genes and genes of signaling systems proteins. Transmission of the signal from regulatory gene to target gene by miRNAs is not limited, since one miRNA can interact with any number of target genes having a binding sites in their mRNAs. It was found that some genes have from one to several dozen binding sites in mRNA. In order to reduce the proportion of these sites in mRNA, the binding sites are clustered. Thus in mRNA region of about 100 nucleotides in length, two to several dozens of binding sites can be located with nucleotide sequence overlap. A great variety of the effectiveness of miRNA-mRNA interaction is achieved because of specificity and selectivity of their interaction, ratio of miRNAs concentrations and concentrations of mRNA relative to miRNA. Competitiveness of miRNA binding to mRNA results in the fact that a more strongly binding miRNA disables the influence of other miRNAs, that is, the effect of regulator genes. Either miRNA presented at a higher concentration competitively eliminates the effect of miRNAs having similar binding characteristics to mRNA of target gene. This effect may change by increased expression of other miRNAs. There are cases of an increase (decrease) in the expression of miRNAs in tens and hundreds of times.

Existing systems of regulation of gene expression suppose their regulation in the cell. Generally, such regulation is represented in form of schemes in which regulator gene (or its product) affects target gene (or its product). This relationship of genes in the regulatory system of genome (gene) expression is not biologically appropriate for a number of reasons. The proteins synthesized in cell, with a few exceptions, do not leave the cell and signaling between genes remains intracellular. Regulatory proteins that left the cell to interact with target genes or target proteins have difficulty penetrating cells containing targets. Therefore, the time of signal transmission of regulator to target is long enough. The transmission of signal from regulatory gene to target gene is limited by ability of proteins to interact with several proteins.

Conclusion. It is shown for the first time that miRNA binding sites can be located in the form of clusters. That is, nucleotide sequences of binding sites of several miRNAs are localized in the mRNA region, which is many times shorter than the sum of nucleotide sequences of all miRNAs. This is achieved by arranged location of miRNAs binding sites with maintaining of high specificity. Such compact localization of miRNA binding sites allows economical using of the nucleotide sequence of mRNA. Since the cluster organization of binding sites is observed in 5'UTR, CDS and 3'UTR, it allows particularly to have such regions that encode not necessarily functionally important oligopeptides of protein in the protein coding region. Because in some cases, binding sites in the CDS encode oligopeptides with significantly different lengths in different species while maintaining a functionally complete protein.

The paper shows that widely used programs for predicting miRNA binding sites in mRNA based on 6-8 nucleotide sites (seed) in miRNA are inadequate, since many false positive sites are predicted. In a comparative aspect, these programs highlight the advantages of the MirTarget program used by us for the prediction of binding sites with quantitative characteristics of the interaction of miRNA with mRNA. A hypothesis of the regulation of expression of genes and genomes involving miRNA is proposed. The role of miRNA as an integrating system for the mutual regulation of gene expression in the cell and in the body is shown.

Authors' contributions. Data for tables 2 were provided by Aisina D.E., for tables 3, 4 and 6 by Kondybayeva A., for table 5 by Yurikova O.Yu. All authors involved in drafting the manuscript, read and approved the final version of the manuscript.

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miRNA: ЖЕТІСТІКТЕР, ПРОБЛЕМАЛАР, ПЕРСПЕКТИВАЛАР

Аннотация. Кіші молекулалы РНК-ның арасында miRNA-дары маңызды рөл атқарады, олар посттранскрипциялық деңгейде гендер экспрессиясын реттейді. Мақалада miRNA-дың қасиеттері мен олардың mRNA-мен өзара әрекеттестігі талқыланады. miRNA-дың түрлі метаболизм процестеріне қатысатын белок-кодтайтын гендердің экспрессиясын реттеуде және жүрек-қан тамырлар, онкологиялық және нейродегенеративті аурулардың дамуындағы рөлі көрсетілген. miRNA-дың нысана гендердің mRNA 5'UTR, CDS және 3'UTR-да байланыстыру сайттарының ерекшеліктері анықталды. mRNA-мен miRNA байланыстыратын сайттарды іздеу белгілі бағдарламаларымен салыстырғанда MirTarget бағдарламасының артықшылықтары көрсетілген. Түрлі аурулардың көптеген кандидатты гендерінің mRNA-ның 5'UTR, CDS және 3'UTR-да бірыңғай miRNA байланысу сайттары және miRNA байланыстыру сайттар кластерлері анықталады. Транскрипция факторларының гендерінің mRNA-ның белоктарындағы олигопептидтерді кодтайтын miRNA байланыстыру сайттар анықталды. miRNA-ның жүрек-қан тамырлар, онкологиялық және нейродегенеративті ауруларға қатысатын кандидатты гендердің mRNA-мен өзара әрекеттесуі талданды. Бірнеше жүздеген гендердің mRNA-да уникалды miRNA-ның байланыстыру сайттар қасиеттері талқыланды. RISC кешенінде mRNA-мен miRNA-ның өзара әрекеттесудің ерекшеліктері қарастырылды. miRNA хост гендерді қамтитын гендердің өзара әрекеттесуі арқылы ген мен геномның экспрессиясын реттеудегі miRNA рөлі талқыланады. miRNA-ды қамтитын ген мен геномның экспрессиясын реттелуінің гипотезасы ұсынылады. miRNA-ның клетка және ағзадағы гендік экспрессиясын өзара реттеу үшін интеграторлық жүйе ретінде рөлі көрсетілген.

Түйін сөздер: miRNA, mRNA, гендер, байланыстыру сайттар, биоинформатикалық бағдарламалар.

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miRNA: ДОСТИЖЕНИЯ, ЗАБЛУЖДЕНИЯ, ПЕРСПЕКТИВЫ

Аннотация. Среди малых RNA важную роль играют miRNA, которые осуществляют регуляцию экспрессии генов на посттранскрипционном уровне. В работе рассмотрены свойства miRNA и их взаимодействие с mRNA. Показана роль miRNA в регуляции экспрессии белок-кодирующих генов участвующих в различных процессах метаболизма и развитии сердечно-сосудистых, онкологических и нейродегенеративных заболеваний. Установлены особенности сайтов связывания miRNA в 5'UTR, CDS и 3'UTR mRNA генов-мишеней. Показано преимущество программы MirTarget перед известными программами поиска сайтов связывания miRNA с mRNA. В mRNA многих кандидатных генов различных заболеваний выявлены одиночные

сайты связывания miRNA и кластеры сайтов связывания miRNA в 5'UTR, CDS и 3'UTR mRNA. В mRNA генов транскрипционных факторов обнаружены полисайты связывания miRNA которые кодируют олигопептиды в составе белков. Анализируются взаимодействие miRNA с mRNA кандидатных генов, участвующих в сердечно-сосудистых, онкологических и нейродегенеративных заболеваниях. Обсуждаются свойства уникальных miRNA имеющих сайты связывания в mRNA нескольких сот генов. Рассмотрены особенности взаимодействия mRNA с miRNA в составе комплекса RISC. Обсуждается роль miRNA в регуляции экспрессии генов и генома посредством взаимодействия генов с участием miRNA хозяйских генов. Предложена гипотеза регуляции экспрессии генов и геномов с участием miRNA. Показана роль miRNA как интегрирующей системы взаиморегуляции экспрессии генов в клетке и организме.

Ключевые слова: miRNA, mRNA, гены, сайты связывания, биоинформатика.

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CO-CIRCULATION OF INFLUENZA A AND B VIRUSES AMONG HUMANS IN THE ARAL REGION OF THE REPUBLIC OF KAZAKHSTAN DURING THE 2015–2017 EPIDEMIC SEASONS

Abstract. In 2015-2017, 2105 biosamples (1978 nasopharyngeal swabs and 127 serums) were obtained from patients in polyclinics and infectious diseases hospitals in Aktobe and Kyzylorda regions of the Republic of Kazakhstan.

Using the polymerase chain reaction for 1978 samples collected from humans, the genetic material of the influenza A virus was detected in 10.86% of cases, that of the influenza B virus in 9.15%. While subtyping influenza A virus RNA, A/H1 subtype was identified in 9.76% of samples, A/H3 subtype in 89.30%. The results obtained from the screening of nasopharyngeal swabs in the polymerase chain reaction, as well as serological data in the hemagglutination inhibition reaction and enzyme immunoassay indicate co-circulation of the A/H1N1, A/H3N2 and B influenza viruses in humans in the Aktobe and Kyzylorda regions of the Republic of Kazakhstan during the 2015-2017 epidemic seasons.

In the virological study of nasopharyngeal swabs obtained from humans, 13 hemagglutination agents were isolated on chick embryos, 10 of which were identified in the hemagglutination inhibition and neuraminidase inhibition assays as influenza A/H1N1 viruses, and 3 as influenza B viruses.

The results from virological and serological studies indicate the need for continuous surveillance of the influenza virus circulation among humans in Aktobe and Kyzylorda regions in order to timely predict epidemic outbreaks and carry out preventive measures.

Keywords: circulation, influenza virus, subtype, isolate, hemagglutinin, neuraminidase, chain polymerase reaction, enzyme-linked immunosorbent assay.

Introduction. Among acute respiratory viral infections, influenza has the greatest clinical and epidemiological importance for humans. Each year about 600 million cases of influenza are registered worldwide; at that, 3 million people suffer from serious diseases that lead to a lethal outcome in 250,000 - 500,000 cases [1].

Since 1890, type A viruses periodically, at intervals of 10 to 40 years, cause pandemics resulting from the emergence of radically new variants of influenza viruses, against which there is little or no immunity in the human population, a process called antigenic shift. The last 2009/2010 influenza pandemic was caused by A(H1N1)pdm09 virus, which contains a complex combination of the gene segments of swine, avian and human influenza viruses. This virus completely replaced circulating earlier seasonal viruses A(H1N1) and continues to circulate around the world together with A(H3N2) and type B viruses [2].

Influenza viruses are the most variable among human viruses due to the high mutation rate, rapid replication, the presence of a segmented genome (which facilitates the gene recombination between different influenza viruses), and cases of the introduction of zoonotic A type viruses [3].

The spectrum of epidemic strains of influenza viruses and their characteristics vary depending on the season of the year. Recently, in Kazakhstan, as in many countries around the world, there is a simultaneous circulation of influenza viruses of the A(H1N1), A(H3N2) subtypes and B genus [4-8].

The purpose of this work was to study the peculiarities of the influenza virus circulation in the Aral region of Kazakhstan during the 2011-2017 epidemic seasons.

Research methods. The collection of clinical samples (nasopharyngeal swabs, serums) from patients was carried out in polyclinics and infectious diseases hospitals during the 2015-2017 epidemic periods in the Aktobe and Kyzylorda regions. The samples were stored in liquid nitrogen before initiation of virological studies.

Primary screening of nasopharyngeal swabs in real-time polymerase chain reaction (RT-PCR) was performed on a RotorGen 6000 amplification system (Corbett Research, Australia) with the RIBO-prep, AmpliSens® Influenza virus A/B-FL and AmpliSens® Influenza virus A type-FL kits (produced by the Central Research Institute for Epidemiology of Rospotrebnadzor, Moscow) [9].

Virus isolation was carried out in two systems using traditional methods: on the MDSC culture with the addition of TRNC-trypsin (2 μg/mL) and 9-11 day-old chick embryos (CE). To indicate the virus in the hemagglutination assay (HA), a 0.75% suspension of the chicken and human 0(1) blood group erythrocytes was used.

The infectious activity of isolates was determined according to the conventional method [10], and their titer was expressed in lg $EID_{50/0.2ml}$ and lg $TCD_{50/0.2ml}$.

Identification of isolates was carried out in the hemagglutination inhibition (HI) and neuraminidase inhibition (NAI) assays with polyclonal diagnostic serum kits according to WHO recommendation [11, 12].

The level of specific antibodies against influenza viruses in serum was determined in HI assay and enzyme-linked immunosorbent assay (ELISA). HI assay was carried out according to the WHO recommendation using both reference viruses A/California/04/09 (H1N1), A/Solomon Islands/03/06 (H1N1), A/USA/1976/31 (H1N1), A/Aichi/2/68 (H3N2), A/Panama/2007/99 (H3N2), B/Florida/04/06, and commercial diagnosticums produced by the FSBI Research Institute of Influenza (St. Petersburg). The test systems intended for influenza viruses of A (H1N1), A (H3N2) subtypes and type B produced by EPDP LLC (Enterprise for the Production of Diagnostic Preparations, St. Petersburg) were used in ELISA.

Results and discussion. The materials were collected during the 2015-2017 epidemic seasons in the medical institutions located in the Aktobe and Kyzylorda regions. In total, 1978 upper respiratory tract swabs and 127 serums were taken from the patients.

More than 90% of the samples were collected from patients diagnosed with acute respiratory viral infection. The greatest number of nasopharyngeal swabs (1291) was obtained from children under 14 years of age (65.27%).

Table 1 shows the characteristics of the collected material and results of the primary RT-PCR based screening of nasopharyngeal swabs.

As can be seen from Table 1, while studying 293 samples collected in 2015, the genetic material of the influenza virus was detected in 40 samples (13.6% of the total number of samples). Influenza A virus RNA was detected in 37 samples (12.6%), that of influenza B virus in 3 samples (1.0%). Subtyping made it possible to detect A/H1N1 virus RNA in 8 swabs (2.7% of cases), A/H3N2 virus RNA in 27 samples (9.2%).

Of 112 samples taken from patients in 2016, the genetic material of the influenza virus was detected in RT-PCR in 18 samples (16.1% of the total number of samples). Influenza A virus RNA was detected in 16 samples (14.3%), that of influenza B virus in two samples (1.8%). Subtyping made it possible to detect A/H1N1 virus RNA in 13 swabs (11.6% of cases), A/H3N2 virus RNA in 3 samples (2.7%).

When examining 1573 biosamples obtained in 2017, the genetic material of the influenza virus was detected in 338 samples (21.5% of the total number of samples). Influenza A virus RNA was detected in 162 biosamples (10.3%), that of influenza B virus in 176 samples (11.2%). Subtyping of PCR-positive samples for influenza A virus revealed the presence of the genetic material of A/H3N2 virus in all 162 samples; it was not possible to detect A/H1N1 virus RNA.

Table 1 – Characterization and RT-PCR based screening of clinical samples collected from humans in 2015-2017

Year	Sampling site	Number of nasopharyn-geal swabs	Number of PCR-positive samples				Number of serums
			for influenza A virus	for viruses of subtypes:		for influenza B virus	
				A/H1N1	A/H3N2		
2015	Aktobe region	39	8	5	1	2	25
	Kyzylorda region	254	29	3	26	1	23
Total:		293	37	8	27	3	48
2016	Aktobe region	17	4	3	2	0	22
	Kyzylorda region	95	12	10	1	2	-
Total:		112	16	13	3	2	22
2017	Aktobe region	768	137	0	137	96	25
	Kyzylorda region	805	25	0	25	80	32
Total:		1573	162	0	162	176	57
Total for 3 years		1978	215	21	192	181	127

Therefore, the primary RT-PCR based screening of nasopharyngeal swabs showed that influenza A and B viruses co-circulated among humans in the Aktobe and Kyzylorda regions in 2015-2017. At the same time, the influenza A/H3N2 virus, which prevailed in 2015 and gave the place to the A/H1N1 virus in 2016, manifested itself again in 2017.

As a result of primary infection and subsequent passages on CE and MDCK cultures, 13 hemagglutinating agents were isolated from PCR-positive samples with titers on CE from 1:32 to 1:1024 and on MDCK culture from 1:4 to 1:32.

Identification of 2015-2017 isolates was carried out in HI and NAI assays. The results of determining the hemagglutinin subtype in the isolates are given in table 2.

Table 2 – Identification of hemagglutinin subtypes for the 2015-2017 influenza virus isolates in HI assay

Isolate	Titer of immune serum antihemagglutinin					
	A/USA/1976/31 (H1N1)	A/Solomon Islands/03/06 (H1N1)	A/California/04/09 (H1N1)pdm	A/Aichi/2/68 (H3N2)	A/Panama/2007/99 (H3N2)	B/Florida/04/06
	1280*	**640**	**640**	**640**	**640**	**640**
Aktobe/02/15	160	160	160	<20	<20	<20
Aktobe /03/15	80	40	40	<20	<20	<20
Aktobe /06/15	80	20	20	<20	<20	<20
Aktobe /18/15	80	20	20	<20	<20	<20
Aktobe /20/15	320	160	160	<20	<20	<20
Kyzylorda/83/15	160	160	160	<20	<20	<20
Kyzylorda /176/16	40	80	40	<20	<20	<20
Kyzylorda /177/16	160	40	20	<20	<20	<20
Kyzylorda /178/16	80	20	20	<20	<20	<20
Kyzylorda/185/16	320	160	160	<20	<20	<20
Kyzylorda /21/17	<20	<20	<20	<20	<20	80
Kyzylorda/28/17	<20	<20	<20	<20	<20	160
Aktobe /73/17	<20	<20	<20	<20	<20	80

*Homologous antibody titers for reference serums are presented; homologous antibody titer for reference serums against A/USA/1976/31 (H1N1) strain was of 1:1280, for the remaining ones of 1:640.

As can be seen from table 2, the hemagglutinating activity of the Aktobe/02/15, Aktobe/03/15, Aktobe/06/15, Aktobe/18/15, Aktobe/20/15, Kyzylorda/83/15, Kyzylorda /176/16, Kyzylorda /177/16, Kyzylorda /178/16, and Kyzylorda/185/16 isolates from 1/32 to 1/4 of the homologous titers was suppressed by immune serums against the A/USA/1976/31 (H1N1), A/Solomon Islands 03/06 and A/California /04/09 (H1N1) pdm viruses. This allowed attributing HAA to the influenza A virus with the H1 hemagglutinin subtype.

The hemagglutinating activity of three isolates (Kyzylorda /21/17, Kyzylorda/ 28/17 and Aktobe /73/17) from 1/8 to 1/4 of the homologous titer was suppressed by immune serums against the influenza B/Florida/04/06 virus. Serums against influenza A/USA/1976/31 (H1N1), A/SolomonIslands/03/06 (H1N1), A/California /04/09 (H1N1) pdm, and A/Aichi/2/68 (H3N2) viruses gave the negative results, which made it possible to classify the 2017 isolates as influenza type B virus.

The results from subtype identification of the second surface glycoprotein for influenza A virus isolates in NAI assay are presented in table 3.

Table 3 – Identification of neuraminidase subtype for the 2015-2016 influenza virus isolates in NAI assay

Isolate	Antibody titer against neuraminidase subtypes	
	N1	*N2*
A/Aktobe/02/15	100	<20
A/Aktobe /03/15	100	<20
A/Aktobe /06/15	100	<20
A/Aktobe /18/15	100	<20
A/Aktobe /20/15	100	<20
A/Kyzylorda/83/15	100	<20
A/Kyzylorda/176/16	100	<20
A/Kyzylorda/177/16	100	<20
A/Kyzylorda/178/16	100	<20
A/Kyzylorda/185/16	100	<20
Note. The reciprocals of antineuraminidase antibody titers are presented.		

It can be seen from Table 3 that the neuraminidase activity of all isolates in titers of 1:100 was suppressed by the immune polyclonal serum against the A/H1N1 virus.

Therefore, according to the results of HI and NAI assays, the 2015-2016 isolates were attributed to influenza A viruses with A/H1N1 antigenic formula, and the 2017 isolates to influenza type B virus.

To evaluate the 2015-2017 seroepidemiological situation of influenza in the Aral region, 127 serums were examined in HI assay and ELISA. The results of HI assay are shown in figure 1.

As can be seen from figure 1, in the 2015 epidemic season, antihemagglutinins to the influenza A/H3N2 virus were detected in human serums in 60.4% (29 samples), in 10.5% of cases (5 samples) the serums were found to be seropositive against the influenza A/H1N1 virus. The serums were positive against influenza B virus in 6.3% of cases (3 samples); antihemagglutinins simultaneously to influenza A/H1N1 and A/H3N2 viruses were detected in 4.2% (2 samples) and to influenza A/H3N2 and B viruses in 2.1% (1 sample). Antibody titers were of 1:80-1:320.

In 2016, antihemagglutinins to the influenza A/H1N1 virus were detected in human serums in 40.9% of cases (10 samples), 18.2% of cases (4 samples) were found to be seropositive against the influenza A/H3N2 virus. In 9.1% of cases (2 serums) antihemagglutinins to influenza B virus were detected.

In 2017, antihemagglutinins to the serotype A/H3N2 virus were detected in 47.4% of cases (27 samples), to influenza type B virus in 5.3% (3 samples); antibodies simultaneously to influenza A (H1N1 + H3N2) viruses were detected in 19.3% of cases (11 samples). Antibody titers were of 1:80-1:320.

Figure 2 presents the results of a serological study of 127 serums in ELISA.

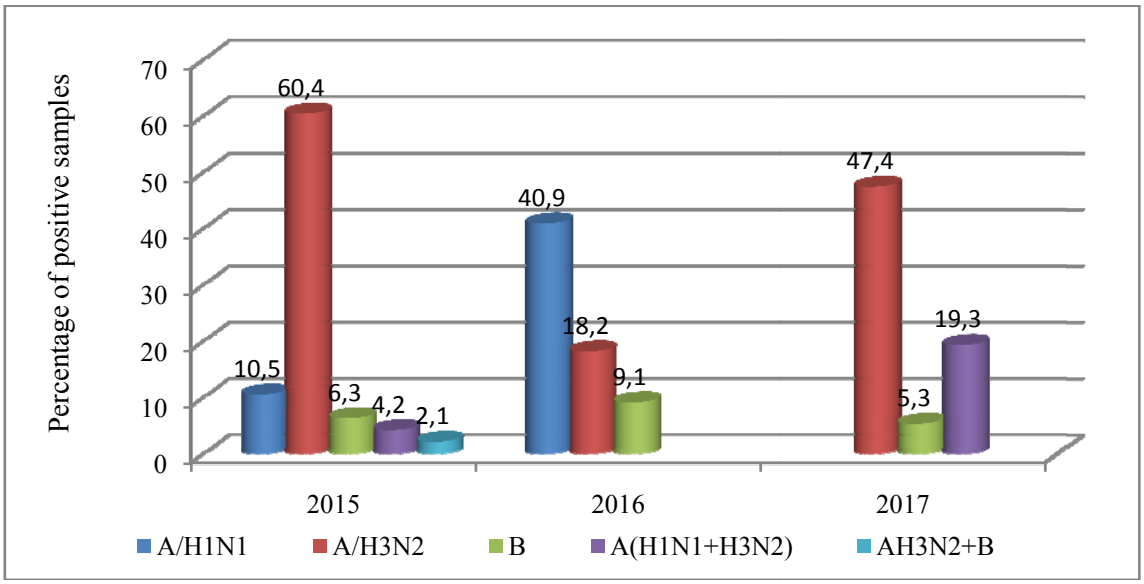

Figure 1 – Detection of specific antibodies against influenza viruses in serums in HI assay

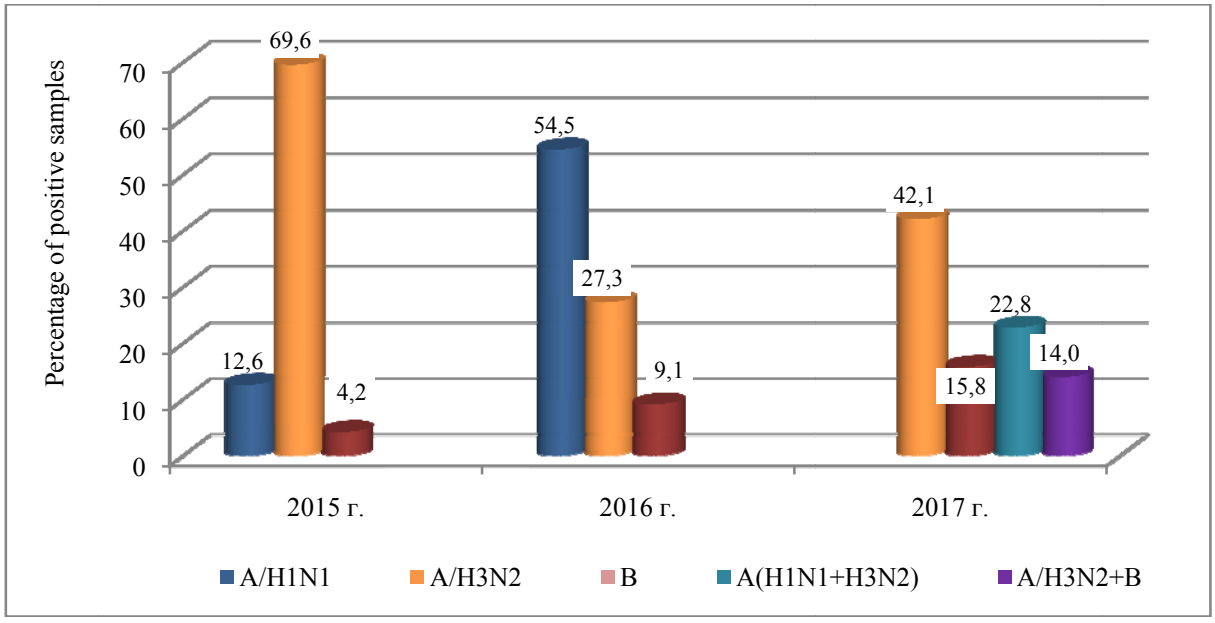

Figure 2 – Identification of antibodies against influenza viruses in serums in ELISA

As can be seen from figure 2, in the 2015 epidemic season, antibodies against the influenza A/H3N2 virus were detected in 69.6% of cases (28 samples), influenza A/H1N1 virus in 12.6% (6 samples), and influenza B virus in 4.2% (2 serums).

While studying 22 serums obtained in 2016, antibodies against the influenza A/H1N1 virus were detected in 54.5% of cases (12 samples), influenza A/H3N2 virus in 27.3% (6 samples), and influenza B virus in 9,1% (2 serums).

In the 2017 epidemic season, antibodies against the influenza A/H3N2 virus were detected in the vast majority of serums (42.1% - 24 samples), influenza B virus in 15.8% of the serums (9 samples); antibodies simultaneously against A (H1N1+H3N2) viruses were detected in 22.8% of cases (13 serums), A/H3N2 and B viruses in 14.0% (8 samples).

Therefore, the results from serological studies of serums in ELISA and HI assay indicate co-circulation of influenza A/H1N1, A/H3N2, type B viruses and mixed influenza infection in the Aktobe and Kyzylorda regions during the 2015-2017 epidemic seasons. A distinctive feature of the 2017 epidemic season is the high content of antibodies against the A/H3N2 and B virus.

According to the literature data, recently there has been a simultaneous circulation of strains representing various evolutionary lines of influenza A and B viruses [4, 13-16]. At that the antigenic composition of the viral population varies depending on the epidemic seasons [17-19]. Subtypes H1N1 and H3N2 of influenza A viruses are widespread among humans. Influenza B virus infection proceeds easier as compared with type A, produces small outbreaks and rare mutations [20].

The unique antigenic variability of influenza viruses, which allows them to overcome interspecies barriers, leads to the emergence of viruses with new biological properties that are capable of wide epidemic spread. [21] In connection with this the most important areas of the fight against influenza include the surveillance of the infection spread, timely pathogen diagnostics, and disease prevention.

Conclusions. In the initial screening of nasopharyngeal swabs and serological studies of serums collected in the 2015 -2017 epidemic season from the patients in Aktobe and Kyzylorda regions, co-circulation of influenza A/H3N2, A/H1N1 and B viruses was established in RT-PCR, HI assay, and ELISA.

As a result of virological studies, ten isolates of influenza A/H1N1 and three isolates of influenza B viruses were obtained from clinical samples, which confirmed the circulation of influenza viruses in the region.

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РМК «Микробиология және вирусология институты» ҚР БҒМ ҒК, Алматы, Қазақстан

2015-2017 ЖЖ. ТҰМАУ ІНДЕТІ АРАЛЫҒЫНДАҒЫ ҚАЗАҚСТАН РЕСПУБЛИКАСЫ АРАЛ МАҢАЙЫ ТҰРҒЫНДАР АРАСЫНДАҒЫ А ЖӘНЕ В ТҰМАУ ВИРУСТАРЫНЫҢ АЙНАЛЫМЫ

Аннотация. 2015-2017 жж. аралығында Ақтөбе және Қызылорда облыстарындағы инфекциялық емханаларымен поликлиникаларындағы сырқат адамдардан 2105 биосынамалар алынды. (1978 танау-мұрын жағындысы және 127 қан сарысуы).

Полимеразды тізбекті реакциясында адамдардан жиналған 1978 үлгіден А тұмау вирусының генетикалық материалы 10,86% жағдайында анықталды, В тұмау вирусы – 9,15%. А тұмау вирусын субтиптеу кезінде А/Н1 тұмау вирусы – 9,76% сынамасында анықталса, А/Н3 – 89,3% құрады.

Мұрын-танау жағындысын полимеразды тізбекті реакциясында скрининг жүргізу және қан сарысуын гемагглютинация тежеу рекциясымен иммуноферментті талдаудағы зерттеу нәтижелері, Ақтөбе және Қызылорда облыстарындағы адамдар арасында 2015-2017 жж. А/Н1N1, А/Н3N2 және В тұмау вирустары айналымда жүргендігін көрсетеді.

Адамдардан жиналған мұрын-танау жағындыларын вирусологиялық зерттеу нәтижесінде, тауық эмбриондарында 13 гемагглютининдеуші агент бөлініп алынды. Нейраминидаз белсенділігін тежеу реакциясы және гемагглютинация тежеу реакциясында 10 А/Н1N1 тұмау вирусы, 3 В тұмау вирусы болып анықталды.

Вирусологиялық және серологиялық зерттеулердің нәтижелері Ақтөбе және Қызылорда облыстарындағы адамдар арасындағы тұмау індетін алдын-ала болжау және профилактикалық іс-шараларды жүргізу үшін, тұмау айналымын үздіксіз қадағалау қажеттілігін көрсетеді.

Түйін сөздер: айналым, тұмау вирусы, типасты, изолят, гемагглютинин, нейраминидаза, полимеразды тізбекті реакция, иммунноферметті талдау.

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СОЦИРКУЛЯЦИЯ ВИРУСОВ ГРИППА А И В СРЕДИ ЛЮДЕЙ В АРАЛЬСКОМ РЕГИОНЕ РЕСПУБЛИКИ КАЗАХСТАН В ЭПИДЕМИЧЕСКИЕ СЕЗОНЫ 2015-2017 ГГ.

Аннотация. В 2015-2017 гг. в Актюбинской и Кызылординской областях РК от больных людей в поликлиниках и инфекционных больницах получено 2105 биопроб (1978 носоглоточных смыва и 127 сывороток крови).

В полимеразной цепной реакции в 1978 образцах, собранных от людей, генетический материал вируса гриппа А был обнаружен в 10,86% случаев, вируса гриппа В – в 9,15%. При субтипировании РНК вируса гриппа А подтип A/H1 идентифицирован в 9,76% проб, A/H3 – в 89,30%.

Результаты, полученные при скрининге носоглоточных смывов в полимеразной цепной реакции, также как и данные серологических исследований в реакции торможения гемагглютинации и иммуноферментном анализе, указывают на социркуляцию вирусов гриппа A/H1N1, A/H3N2 и В у людей в Актюбинской и Кызылординской областях РК в эпидемические сезоны 2015-2017 гг.

При вирусологическом исследовании носоглоточных смывов, полученных от людей, на куриных эмбрионах выделено 13 гемагглютинирующих агентов, 10 из которых идентифицированы в реакции торможения гемагглютинации и реакции ингибиции нейраминидазной активности как вирусы гриппа A/H1N1, три – как вирусы гриппа В.

Результаты вирусологических и серологических исследований свидетельствуют о необходимости проведения постоянного надзора за циркуляцией возбудителей гриппа среди людей в Актюбинской и Кызылординской областях с целью своевременного прогнозирования эпидемических вспышек и проведения профилактических мероприятий.

Ключевые слова: циркуляция, вирус гриппа, подтип, изолят, гемагглютинин, нейраминидаза, цепная полимеразная реакция, иммунноферметный анализ.

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THE GENETIC NATURE OF MUTATIONAL CHANGES ARISING IN THE FORM-FORMATION PROCESS OF WHEAT

Abstract. Increasing the yield of wheat by improving its genotype is one of the most pressing problems of agriculture and the economy. Currently, the usage of traditional breeding methods and the results of genetic investigations, such as conduction of saturating crosses, remote hybridization and experimental mutagenesis, increase the efficiency of producing genetically modified and enriched forms of wheat. In field and under controlled laboratory conditions, the effect of a surfactant on the heritable characteristics of 10 varieties of spring soft wheat was studied. After processing of wheat seeds with an aqueous surfactant solution (0.1%), we could observe the inherited changes, which are manifested in the appearance in M_1, M_2, $F_2$and BC_1 of tall, potent plants with productive bushiness and various morphological characteristics that differ from the original varieties. The effect of surfactants is manifested on the morphological features of plants: bushiness, crankiness of the stem, anthocyanin stain color. Duringthe process of meiosis, the spindle of the division of the metaphase plate, the coalescence of chromosomes in MI, and the presence of empty (sterile) cells in AI and AII meiosis were observed. The signs of altered forms are stably transmitted in the M_2 generation.

Key words: selection, chemical mutagenesis, variety.

Mutational selection involves the development of new varieties by creating and using genetic variability through chemical and physical mutagenesis [1-5]. Completely new forms such as dwarf mutants of wheat and barley, superfast mutants in barley, plants resistant to fungal diseases, highly productive mutants serving as precursors of new high-yielding varieties, are obtained as a result of chemical mutagenesis [6-12]. However, obtaining mutants and studying them is only the first stage of breeding work. In the selection of mutations, hybridization can be used. It is more important to use the mutants in hybridization to obtain positive transgressions.

The preparation of mutants and their use for hybridization requires studying the genetic nature of the changes which occur in living cells, which is crucial for the selection of effective mutagens with a specific effect, and for expanding and deepening understanding of the nature of wheat evolution. To increase the efficiency of mutational plant breeding and the yield of appropriate mutations, it is essential to study the conditions and methods of the mutagenicprocess that allow expanding the spectra of hereditary variability. Induced hereditary changes (mutations) caused by physical and chemical mutagens are random and cannot be controlled. For example, a high mutagenic activity of ethylenimine, diethyl sulfate and dimethyl sulfate is shown on a number of specimens of peas and beans. It was shown that with increasing the mutagen concentration the incidence of mutations increases too, but most mutants did not represent a breeding value [13-18].

Of great importance is the problem of studying genetic effects and, in particular, the specificity of changes in mutations caused by the modifying effects of environmental conditions (certain fertilizer doses [19-22], the effects of nicotinic acid of natural origin) that caused certain changes - the emergence of powerful tall plants, the so-called large genotrophs [2]. The difference in size persisted in subsequent generations. Such changes, according to Waddington, are called "epigenetic mutations." "Epi" in Greek means "outside", "near", i.e. differences that occur somewhere near the genes, near them, but not in themselves genes [12].

In recent years, the attention of researchers has attracted the use of surfactants in various fields of science (medicine, agriculture, etc.).

It is shown that surfactants have not only bactericidal activity, but also the ability to enhance the action of various antibiotics. In the culture of fibroblasts, surfactants disrupt ion homeostasis; stimulate the synthesis of DNA and proliferation of the cell monolayer [15].

P.I. Kudinov and T.V. Karaim conducted studies that showed the inhibitory effect of surfactants (methacide) on the bacteria of the potato bacillus group when processing wheat grain; they also found the optimal dosage of metacid for grain processing.

We used a surfactant, which was obtained on the basis of a plant of camel thorn. Employees of the Department of Organic Chemistry of the Chemical Faculty of KazNU named after al-Farabi determined the polyphenolic composition of this plant and revealed biologically active substances showing physiological activity. The drug, called alchidine - polymer proanthocyanidin is non-toxic. The conducted studies showed a high degree of inhibition of cell division during the action of alchidine on malignant neoplasms. Based on surfactant (alchidine), an antitumor drug was obtained. This drug was also used to preserve eggs in the Research Institute of Fishery (Astrakhan), while the content of vitamin E was increased by 2 times.

Because of the treatment of wheat seeds with a surfactant (alchidine), we obtained powerful plants with high productive bushiness, thick straw, as well as plants with long ears and elongated scales having large vitreous grains. The mechanisms of the damaging development or changing the quantitative and qualitative characteristics of plants under the influence of surfactants have been little studied and require further investigation.

Materials and methods

The material of the study was the plants M1, M2, obtained from the treatment with a surfactant of 10 regionalized varieties of spring soft wheat (Shagala, Tolkyn, Dauyl, Kazakhstan 17, Kazakhstan 4, Kazakhstan 3, Zhenis, Lutescens 32, Aray, Kazakhstan 10), as well as offspring of hybrids F1, BC1 from recurrent crossing of altered plants with initial varieties.

The treatment was carried out by soaking the seeds with a 0.1% surfactant solution for 5 hours, at a temperature of 25-27 °C. Control was dry wheat seeds. Surface treated M1 seeds were seeded in duplicate for 100-200 pieces. Selection of the modified plants was carried out in M2. The proportion of the changed plants was taken into account by different characters from the total number of planted plants. Plants with altered traits in M2 were again sown to produce M3 progeny.

Genetic analysis of the modified plants M2 was carried out by crossing them with the original varieties. The analysis of the progeny of F1 hybrids from reciprocal crossing, as well as F2 hybrids and BC1. In experiments were used the following research methods: cytogenetic, hybridological, statistical and morphological. Cytological studies carried out in temporary squash preparations, using microscope LOMO Mikmed-1. Genetic analysis of hybrids F1 and F2 was carried out according to qualitative and quantitative traits of wheat. Statistical data processing was to find the arithmetic mean and its error for the analyzed quantitative traits and definition of the reliability of differences between the arithmetic with the help of student's criterion (t), genetic – finding accurate values of $\chi 2$ [12]. Accounting of chromosomal abnormalities in MI, AI and ALL of meiosiswas carried out on time acetocarmine preparations under the microscope MBI-3. The representativeness of the research results ensured a sufficient sample size – 60 to 100 plants.

Mathematical processing of data was performed by finding the arithmetic mean and its error for the analyzed quantitative traits and to evaluate the accuracy of the difference.

Results and discussion

Morphological changes of plants under the action of surfactants. The study of the effect of surfactants on regionalized varieties of spring soft wheat (Shagala, Tolkyn, Dauyl, Kazakhstan 3, Kazakhstan 4, Kazakhstan 17, Zhenis, Lutescens 32) showed that the effect of surfactants leads to various morphological changes in plants, expressed in stimulating germination, accelerating the growth of primary cornea and the subsequent increase in the productivity of plants. The modified plants were

distinguished by increased bushiness, in comparison with the control (by 3-4 stems), higher and thicker straw, thickening and elongation of stem nodes, lengthening of the joints of the rod, anthocyanin color of the straw and coleoptiles, and a larger grain. Morphological changes in the spike were expressed in the appearance of plants with a supra, speltoid, multiflorous, compactoid, branchy, friable and long spike. At the same time, plants with fragile ears and thin straw were found. In some varieties of the experimental variant, a wide range of variability in plant height was noted. All these changes in the quantitative and qualitative traits of wheat may be related to epigenetic changes.

In the experimental variant of Kazakhstanispecimen 3, a large variation of the spike types was observed, and it proved to be the most susceptible to the action of surfactants.

Among a variety of altered forms, plants with elongated ears, with long scales and glassy elongated grains, have been selected that are resistant to different types of rust, which is important for breeding for resistance.

For example, varieties Dauyl, Lutescens 32, Zhenis and Shagalawere distinguished by their high bushiness, elongated spike, extended form of internodes.

Table 1 shows the data on the elements of productivity of varieties under the influence of surfactants

Variants Average Height (cm) Productive bushiness	Variants A verage				
	Height (cm) Productive bushiness	Height (cm) Productive bushiness	Height (cm) Productive bushiness	Height (cm) Productive bushiness	Height (cm) Productive bushiness
Aray - K	103,7 ± 0,7 99,9**± 1,6	6,8 ± 0,6 10,6*** ± 0,9	11,0 ± 0,2 9,8***± 0,4	43,0 ± 0,8 48,0 ± 0,2	1,3 ± 0,2 1,5 ± 0,1
Anexperience	117,0 ± 1,1 110,0**± 1,9	6,8 ± 0,7 10,5***± 0,1	11,1 ± 0,7 7,8** ± 1,3	41,7 ± 0,5 49,7* ± 1,0	1,7 ± 0,2 1,4 ± 0,3
Daouil-K	103,5 ± 0,5 101,2 ± 1,8	7,3 ± 0,4 10,7* ± 1,3	10,8 ± 0,1 10,7 ± 0,8	45,5 ± 1,0 39,6***± 0,4	1,6 ± 0,5 1,0 ± 0,4
Anexperience	99,3 ± 0,8 102,1* ± 1,1	4,8 ± 0,6 12,0*** ± 0,3	12,3 ± 0,8 10,5 ± 1,1	33,0 ± 0,8 38,3*** ± 0,2	1,9 ± 0,6 1,2 ± 0,3
Chagall - K	107,2 ± 0,3 106,6 ± 0,4	10,5 ± 0,9 10,6 ± 1,5	11,5 ± 1,3 16,8*** ± 0,4	38,0 ± 0,1 36,0 ± 0,9	1,3 ± 0,2 1,2 ± 0,3
Anexperience	79,0±0,2 79,4±1,1	9,3 ± 1,0 10,5 ± 1,6	6,3 ± 1,6 10,4** ± 1,9	34,5 ± 0,7 37,6***± 0,3	1,1± 0,1 2,8***± 0,3
Zhenis - To	97,5 ± 0,8 102,3** ± 0,1	10, 7 ± 0,5 9,6 ± 0,5	8,2 ± 0,3 9,6 ± 2,2	38,4 ± 0,7 37,8 ± 0,7	1,1 ± 0,1 1,8 ± 0,6
Note: *atP > 0,95; 2.**atP > 0,99; ***atP > 0,999; K – Control.					

As can be seen from Table 1, surfactant significantly reduces the height of plants of the following varieties: Arai by 3.8 cm, Dauyl by 7.0 cm, Shagala by 2.3 cm, Zhenis by 2.8 cm. With a significant decrease in the average height of plants in these varieties significantly increases their productive bushiness 10.6 ± 0.9; 10.5 ± 0.1; 10.7 ± 0.3; 12,0 ± 0,3 in comparison with the control 6,8 ± 0,7; 7.3 ± 0.4 and 4.8 ± 0.6, respectively. In Kazakhstan 3 and Lutescens varieties 32 differences in plant height between control and trial variants were not observed.

In the grade of Kazakhstan 10, the stalk was 4.8 cm longer than the control.

Mass of grain from the main ear. With the action of surfactants in almost all studied varieties, the amount and mass of grain from the main spike remain at the control level. The exception is grade Kazakhstan 3, where there was a significant increase in both the number of grains (by 3.1 grains) and the weight of grain from the main ear (1.65 g) compared to the control. In this case, there was a specific reaction of the genotype – Kazakhstan 3 to the effect of surfactants. At the same time, there was a tendency to increase all the studied features of Kazakhstani variety 3, except for plant height, which remains at the control level.

To study the inheritance of morphological characters of Kazakhstani variety 3, a reciprocal crossing was performed between the altered plants M1 and the initial variety. The initial grade Kazakhstan 3 does not have a pubescent ear, anthocyanin stalk color and an elongated form of the cauline node, and in some plants M1, these features are evident.

Table 2 – Reciprocal crossing of modified plants of Kazakhstan variety 3 with initial variety

Symptoms	Kaz 3	M_1	K3 x M_1	M_1 x Kaz.3
Spout of the ear	not pubescent	pubescent	pubescent	pubescent
Staining of stalks	не окрашен	painted	painted	painted
Shape of caulinenodes	normal	elongate	elongate	elongate
Height of plants	79,0±1,2	86,0***±0,8	86,4±0,2	88,3±0,3
The length of the main ear (cm)	9,3 ± 0,6	12,3**±0,7	13,3±0,3	13,9±0,3
Number of grains with Ch. ear	34,5 ± 0,7	39,0 ± 0,6	41,5 ± 1,1	43,7 ± 1,1
Grainweightfromgl.	1,0±0,1	2,7±0,3	3,0± 0,8	3,7 ± 0,5

As can be seen from table 2, the altered morphological features manifest themselves irrespective of the direction of crossing. This indicates a possible inheritance of these features in the succeeding generations M2 and... Mn.

The results of the studies showed that the reaction to the action of the surfactant depends on the genotype of wheat. The variability found in M1 for a number of quantitative and qualitative characteristics persisted in the subsequent generation of M2. This was confirmed by the results of the analysis of the crossing and analysis of the M2 progeny. The presence of altered forms with positive signs: short-stemmed plants with powerful, multiflorous, pubescent ears; plants differing in length and shape of the main ear; by the color, shape and size of the grain can be considered as confirmation of the presence of a

Table 3 – Metaphase I in Controlled and Modified M1 Plants under the Influence of Surfactants

Variant	Number of cells studied		Percentageofcells					
	all	cells with impaired	violations	univalent	open bivalents	pycnosis	dislocation of metaphase plates	polyvalent
Kaz 3 –К	159	32	20	–	12	–	–	–
experiment	164	78	47	4	12	30		
Kaz 10–К	153	16	10	9	–	–	1	–
experiment	185	113	61	12	21	8	18	
Tolkyn –К	166	20	12	2	4	2	4	–
experiment	156	129	82	15	14	25	26	
Aray–К	155	15	8	–	8	–	–	–
experiment	151	46	30	15	5	1	7	
Kaz 17–К	175	18	13	6		3		6
experiment	225	167	74	16	13	22	22	
Dauyl –К	162	12	14		8	–	–	–
experiment	195	49	25	4	12	1,5	4	
Kaz 4–К	156	15	9	–	3	–	6	–
experiment	151	70	46	8	11	14	11	
Zhenis –К	155	19	12	2	9	–	–	
experiment	154	80	51	10	22	18		1
Lut. 32 –К	168	15	8	8				
experiment	152	68	44	16	5	9	11	1
Shagala –К	172	16	9	11				
experiment	178	161	90	24	22	28	15	

Note: К – control variant; experience – experienced.

gene-regulator, which underwent epigenetic changes and, in turn, influences the expression of the registered genes. However, for a simultaneous change in the characteristics of mutants that are different among themselves, the same gene regulator cannot respond. The change that we observe is most likely a consequence of the change in some general processes in the cell that arise in response to the effect of the surfactant.

The effect of surfactants on cell division. Chromosomal aberrations and cell division disorders are one of the main tests for mutagenicity in certain exposures. The most revealing in this respect is the meiotic division of cells, especially in objects such as wheat, which have a large number of hard-to-identify chromosomes.

The main phases on which meiosis is disturbed are metaphase, anaphase of the first division and tetrad. Metaphase I observed such types of disturbances as univalent, polyvalent, open bivalents, chromosome adherence - "pycnosis" and displacement of the spindle of division of the metaphase plate (table 3).

The changes observed in MI were accompanied by a violation of cell division in AI. In this phase, fragments of chromosomes, bridges, asynchronous fission, empty cells were observed. At the level of tetrads - cells with microkernels (table 4). All these changes are a manifestation of violations that occurred at earlier stages, mainly in interphase and early prophase.

Table 4 – Anaphase I in Controlled and Modified M1 Plants under the Influence of Surfactants

Variants	Number of cells studied		Percentageofcells					
	all	cells with impaired	violations	univalent	open bivalents	pycnosis	dislocation of metaphase plates	polyvalent
Kaz 3 –K	156	17	10,8	3,2	1,2	6,4		
experiment	154	66	42,8	17,8	12,9			13,6
Kaz 10–K	167	11	6,5	3,0		3,5		
experiment	158	30	18,9		3,1	15,8		
Tolkyn –K	165	6	3,6	0,6		3,0		
experiment	151	52	34,4	9,9	1,3	19,8	3,3	
Aray–K	159	15	9,4			9,4		
experiment	158	71	44,9	5,0	3,1	6,3	14,5	15,8
Kaz 17–K	167	10	5,9		5,9			
experiment	156	84	53,8	2,5	12,8	19,2	1,2	19,2
Dauyl –K	187	14	8,8	3,2	4,8	4,4		
experiment	154	93	60,0	6,4	22,7	20,7		
Kaz 4–K	171	5	2,2	1,5	4,0	3,6		
experiment	158	68	43,0	6,3	5,0	8,0	3,0	20,0
Zhenis –K	153	13	8,4	5,2	1,3	2,0		
experiment	156	75	48,0	16,0	6,4	3,0		20,0
Lut. 32 –K	157	4	2,54	1,9		0,6		
experiment	152	64	42,0	17,0	1,3	2,6	1,9	11,8
Shagala –K	161	10	6,0	0,6	0,6	4,0		
experiment	156	65	40,0	8,0	3,0	5,0	7,0	17,0

So the maximum percentage of cells with univalentswas found in the experimental variants of Kaz 10 (12%), Tolkyn and Aray (15%), Kaz 17 (16%) and Shagala (24%).

In the control grade of Kazakhstani specimens, 10 cells with univalents made up only 3%, Tolkyn 2%, Kazakhstan 17 and Shagala - 6%. In the remaining studied varieties, the disturbance ranged from 4% to 11%, and in control variants from 1% to 6%.

A high percentage of cells with open bivalents was found in the varieties Zhenis- 34% and Shagala-40%, and in control 9% and 5%, respectively. Pictures, such as open bivalents and univalents can be associated with chromosomal rearrangements that violate the complete homology of chromosomes.

One of the frequent violations in the treatment of SAW seeds was the adhesion of chromosomes (pycnosis). A high percentage of such cells was found in the experimental variants: varieties Kazakhstani 3 (30%), Tolkyn (25%), Kaz 17 (22%). Zhenis (18%) and Shagala (28%). In control variants, cell picnosiswas not detected. The adhesion of chromosomes occurs if the replication of chromosomes is disrupted in the interphase of pre-meiotic division.

Cells with displacement of spindle of division of metaphase plate are found in all varieties, except for cultivars Kazakhstani 3, Zhenis and Lutescens 32, in others the percentage of such cells was from 4% to 26%, and in control variants, such violations were absent.

Cells with a polyvalent configuration of chromosomes were found only in varieties Kazakhstani 17 (6%) and Zhenis (0.6%). In the control variants, these disorders were not detected. In wheat, as a rule, polyvalents are a consequence of conjugation of homologous chromosomes and this occurs with an extension of the conjugation time in the stage of diplotenes. As is known, the chromosome 5B corresponds to this process in wheat.

A comparative analysis of cell damage in different wheat varieties in Metaphase I and Anaphase I meiosis in fractions is given in table 5.

Table 5 – Proportion of cells with disorders in Metaphase I and Anaphase I under the action of surfactant

Variants	Cells with disorders of Metaphase I	Cells with disorders of Anaphase I
Kaz. 3 control	0,8	0,10
experiment	0,47***	0,42***
Kaz. 10 control	0,10	0,06
experiment	0,61***	0,18
Tolkyn control	0,12	0,03
experiment	0,82***	0,34***
Aray control	0,28	0,09
experiment	0,30	0,44***
Kaz. 17 control	0,13	0,05
experiment	0,74***	0,53***
Dauyl control	0,6	0,5
experiment	0,25***	0,60***
Kaz. 4 control	0,09	0,2
experiment	0,46***	0,43***
Zhenis control	0,12	0,08
experiment	0,51***	0,48***
Lut. 32 control	0,08	0,02
experiment	0,44***	0,42***
Shagala control	0,09	0,06
experiment	0,90***	0,40***

As can be seen from Table 5, the proportion of cells with disorders in MI meiosis in Kazakhstan 3 was 0.47, and in control 0.8; Kaz.10 - 0.61 and 0.10; Tolkyn - 0,82 and 0,12; Aray - 0.30 and 0.28; Kaz.17 - 0.74 and 0.13; Dauyl - 0.6 and 0.5; Kaz. 4 - 0.46 and 0.09; Zhenis - 0,51 and 0,12; Lut. 32 - 0.44 and 0.08; Shagala - 0.90 and 0.38.

In the experimental variant in Meiosis AI in the Kazakhstanistrain 3, the proportion of cells with impairments was 0.42, and in control 0.10; Kaz.10 - 0.18 and 0.06; Tolkyn - 0.34 and 0.03; Aray - 0.44 and 0.09; Kazakhstan 17- 0.53 and 0.05; Dauyl - 0.60 - 0.28; Kazakhstani 4 -0.43 and 0.2; Zhenis - 0,48

and 0,08; Lutescens32 - 0.42 and 0.02; Shagala - 0.40 and 0.06. As can be seen from table 5, the proportion of violations in A1 in the pilot variants is much higher than in the control.

The carried out researches have shown the reliable influence of surfactants on quantitative and qualitative attributes of wheat. The surfactant causes an increase or decrease in some of the productivity elements of the altered plants as compared to the control variety. The changed signs were inherited in M2. This is confirmed by the phenotypic manifestation of these features in F1 (BC1) hybrids, in reciprocal crosses. The abnormalities detected in MI, AI and tetrads indicate the effect of surfactants on the meiosis process. As is known, violations occurring before meiotic division are more often transmitted to the next generation.

Thus, the obtained results of the study can have applied value, since under the influence of surfactants, along with suppression of plant development, altered forms with enhanced viability were revealed. The study also found that the reaction of wheat plants to the effect of surfactants depends on the genotype of the studied wheat varieties. The changes observed by us could be a consequence of some general processes in the cell that arise in response to the effect of surfactants. This requires studying the effect of surfactants at the molecular-genetic level. It is planned to conduct chromosomal localization of genes that control the elongation of the ear of wheat, the extension of wheat grain, productive bushiness, anthocyanin color of grain and anthers in the modified plants M3 in Kazakhstani 3. The line obtained, with the above-inherited traits of this variety, can serve as a source of signs of wheat productivity.

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ТҮРТҮЗІЛУ ҮРДІСІНДЕ ПАЙДА БОЛАТЫН МУТАЦИЯЛЫҚ ӨЗГЕРТКІШТІКТІҢ ГЕНЕТИКАЛЫҚ ТАБИҒАТЫ

Аннотация. Ауыл шаруашылығы мен экономиканың ең өзекті мәселелерінің бірі бидайдың генотипін жоғарылату арқылы астық өнімділікті арттыру болып табылады. Қазіргі уақытта асыл тұқымды және генетикалық зерттеулердің дәстүрлі әдістерін қолдану, қанықтырушы шағылыстыру сияқты, алыстан будандастыру және эксперименттік мутагенез, бидайдың генетикалық түрлендірілген және жетілдірілген түрлерін алу тиімділігін арттырады. Далалық және зертханалық жағдайларда беттік белсенді заттың (ББЗ) жазғы жұмсақ бидайдың 10 сорттарының тұқым қуалаушылық белгілеріне әсері зерттелді. Бидай тұқымын судағы беттік белсенді ерітіндісімен (0,1%) өңдеген кезде тұқым қуалаушылық өзгерістер индуцирленіп, M_1, M_2, F_2 және BC_1 жоғары өнімді өсімдіктермен және әр түрлі морфологиялық өзгертілген таңбаларымен ерекшеленеді. Беттік белсенді заттардың әсері өсімдіктердің мынандай морфологиялық ерекшеліктерімен көрінеді: бұтақтылық, сабақтың шөгуі, антоцианин бояуы. Мейоз үрдісін зерттеуде метафаза пластинасының бөліну ұршығы, МІ-дегі хромосомалардың бірігуі, сондай-ақ АІ және АІІ мейоздарындағы бос (стерильді) клеткалардың болуы анықталды. Өзгертілген түрлердің белгілері M_2 ұрпағында тұрақты түрде беріледі.

Түйін сөздер: селекция, химиялық мутагенез, сорт.

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ГЕНЕТИЧЕСКАЯ ПРИРОДА МУТАЦИОННЫХ ИЗМЕНЕНИЙ, ВОЗНИКАЮЩИХ В ФОРМООБРАЗОВАТЕЛЬНОМ ПРОЦЕССЕ ПШЕНИЦЫ

Аннотация. Повышение урожайности пшеницы путем улучшения ее генотипа является одной из наиболее актуальных проблем сельского хозяйства и экономики. В настоящее время использование традиционных методов селекции и генетических исследований, таких как проведение насыщающих скрещиваний, отдаленная гибридизация и экспериментальный мутагенез, повышает эффективность получения генетически модифицированных и улучшенных форм пшеницы. В полевых и лабораторных условиях изучали влияние поверхностно-активного вещества (ПАВ) на наследуемые признаки 10 сортов яровой мягкой пшеницы. При обработке семян пшеницы водным раствором ПАВ (0,1%) индуцируются наследуемые изменения, которые выражаются в появлении в M_1, M_2, F_2 и BC_1 высокорослых, мощных растений с продуктивной кустистостью и различными морфологически измененными признаками, отличающихся от исходных сортов. Действие ПАВ проявляется на морфологических признаках растений: кустистость, коленчатость стебля, антоциановая окраска стебля и листовой пазухи. При изучении процесса мейоза обнаружены смещение веретена деления метафазной пластинки, слипание хромосом в МІ, а также наличие пустых (стерильных) клеток в АІ и АІІ мейоза. Признаки измененных форм стабильно передаются в поколении M_2.

Ключевые слова: селекция, химический мутагенез, сорт.

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ANALYSIS OF *MYOCILIN (MYOC)* AND *NEUROTROPHIN 4 (NTF4)* GENES IN PATIENTS WITH GLAUCOMA IN KAZAKHSTAN

Abstract. Primary Open-Angle Glaucoma being the most common type of glaucoma has a great socio-medical significance and it is a major focus for scientific researching in ophthalmology. In 21-50% of cases the disease is the result of genetic causes and for descendants of people with glaucoma, the risk of this pathology is 10 times higher. Mutations causing Primary Open-Angle Glaucoma have been identified in *MYOC/TIGR* gene, which encodes a 57kDa protein known as myocilin and in the gene *NTF4* that codes a dimeric peptide (28kD). In this study we investigated the frequency of mutations in *MYOC/TIGR* and *NTF4* genes in Kazakhstan population. The study was conducted involving 85 patients diagnosed with primary glaucoma and 100 individuals as a control group. The results of our research show that T353I, D208E in *MYOC/TIGR* gene and R206W polymorphisms in *NTF4* gene among Kazakhstan population has no influence on the POAG progression while R76K SNP in *MYOC/TIGR* gene can be as a genetic factor which affects the development of POAG type of glaucoma.

Keywords: POAG, polymorphism, *MYOC/TIGR*, *NTF4*.

Introduction. Glaucoma is a neurodegenerative disease which is characterized by progressive damage to ganglion cells, optic nerve fibers, and visual field defects. It is one of the main reasons of irreversible blindness in the world. Primary Open-Angle Glaucoma (POAG) is a basic form of primary glaucoma. Glaucoma is a treatable disease if it is detected early, however, many patients get being diagnosed only after the loss of visual field, since glaucoma is typically asymptomatic at the early stages [1].

An estimated 66.5 million people were identified as having open-angle and angle-closure glaucoma by 2010 and it tends to reach 79.6 million by 2020. Binocular blindness in 2010 was observed in 8.4 million patients with glaucoma, and by 2020 it is going to rise to 11.2 million [2]. According to J. Goldberg's estimates, the number of glaucoma patients will reach 120 million by 2030 [3]. Since 2011, 24 750 patients with glaucoma have been registered in Kazakhstan [4].

Primary open-angle glaucoma (POAG) is described distinctly as a multifactorial optic neuropathy that is progressive, and irreversible, with a characteristic acquired loss of optic nerve fibers. POAG is a chronic disease. It may be hereditary. Genetic predisposition is a distinctive feature of primary glaucoma and confirmed in 50% of cases. Currently, there are 4 causative genes and 70 candidate genes associated with the development of POAG [5]. The well-recognized genes associated with POAG include *myocilin* (*MYOC/TIGR*) [6, 7], *optineurin* (*OPTN*) [8] and *neurotrophin-4* (*NTF4*) [9]. In the past 2 years, large scale genetic studies that have examined the blood samples of thousands of glaucoma patients have been instrumental in the discovery of more common genetic risk factors for POAG. For glaucoma, these genetic factors include changes in the DNA sequences or actual loss of DNA, and several different genes have been implicated [10]. How these genes cause or influence the likelihood of developing POAG is of major interest. The definition of a mutation in these genes is important for the diagnosis of glaucoma and genetic counseling of patients.

MYOC/TIGR gene located in chromosome 1q24.3 and expressed in many ocular tissues, including the trabecular meshwork. Therefore, the alternative name for this gene is *TIGR* (*gene trabecular mesh-*

work-induded glucocorticoid response protein). The gene has 3 exons of size 604, 126, and 782 bp. *MYOC* is expressed as a 2.3 kb transcript and the translated product is predicted to contain 504 amino acids (58 kDa) [11]. Myocilin mutations, in general, are more strongly associated with POAG and JOAG than other forms of glaucoma [12, 13].

The next gene is neurotrophin (*NTF4*). Cytogenetic Location: 19q13.33, which is the long (q) arm of chromosome 19 at position 13.33. *NTF4* is translated as pre-pro-neurotrophin. The gene is organized in 2 exons and encodes a polypeptide of 210 amino acids. Neurotrophin protein is dimeric polypeptide with a molecular weight of 28 kDa and they are important regulators of neural survival, development, function, and plasticity. *NTF4* gene is expressed in most parts of the brain and in other tissues [14].

Mutations in the *MYOC/TIGR* and *NTF4* genes result in damage to actin fibers in the trabecular meshwork [15] and a decrease in neurotrophin signal [16]. Mutations in these genes are responsible for the development of glaucoma from 2% to 20%.

The main goal of this study is to investigate the polymorphism of *MYOC/TIGR* (rs772312298, rs2234926, D208E) and *NTF4* (rs121918427) genes in patients with glaucoma in population of Kazakhstan.

Materials and methods. The study was conducted involving 85 patients diagnosed with primary glaucoma. These materials were collected in the Kazakh Research Institute of Eye Diseases and in the Medical Centre Hospital of President's Affairs Administration of the Republic of Kazakhstan. As a control group, people were selected who did not suffer from this disease and they were chosen depending on the age, gender and ethnic composition of patients with glaucoma. Genomic DNA was extracted from 200 µl of whole blood using a kit (*ThermoFisher Scientific,* USA). The concentration of the DNA molecule was determined using a DNA fluorometer (*BioPhotometer plus*, *Eppendorf*, Germany), and the quality was determined by agarose gel electrophoresis.

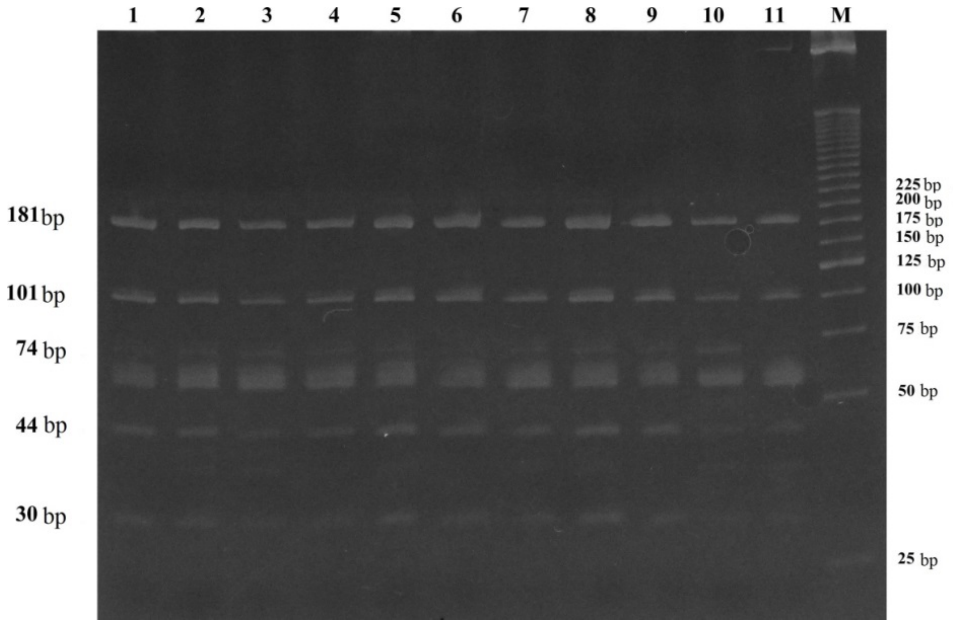
Genotyping of polymorphisms was carried out by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The volume of the reaction mixture for PCR is 20 µl: 50-100 ng of genomic DNA, 5 pmol of primer and Master Mix (*ThermoFisher Scientific,* USA). For the PCR thermal cycle, a touchdown annealing temperature of 62°C minus 0.2°C per cycle for 35 cycles was used in a thermal cycler (*Mastercylcer nexus, Eppendorf,* Germany). Patients with POAG and members of the control group were also screened by restriction analysis. Restriction enzymes were mixed with each sample and incubated with their corresponding buffers overnight at 37°C (*ThermoFisher Scientific,* USA). Primer pairs and restriction enzymes are listed in table 1. DNA fragments were detected by electrophoresis on 2% agarose or 12% polyacrylamide gels.

Table 1 – Primer pairs and restriction enzymes for PCR-RFLP analysis

Polymorphism	Primer pair (5′→ 3′)	Codon changes	Nucleotide changes	Restriction enzyme
T353IR: T353IF:	GCTACCCTTCTAAGGTTCACATAC ATTGGCGACTGACTGCTTAC	$Thr^{353}Ile$	1058 (C→T)	*Hpy*CH4III
R76KR: R76KF:	CTTCTGTGCACGTTGCTGCA CTGGTCCAAGGTCAATTGGT	$Arg^{76}Lys$	227 (G→A)	*Bsm*AI
D208ER: D208EF:	CATAGTCAATCCTTGGGC CTGCAGACCTGCTCTGACAA	$Asp^{208}Glu$	624 (C→G)	*Bsm*AI
R206WR: R206WF:	CCGGAGTCTGCATTTCTTAGT GAAGGAGGCTGGAAGAGATTAC	$Arg^{206}Trp$	616 (C→T)	*Apa*I

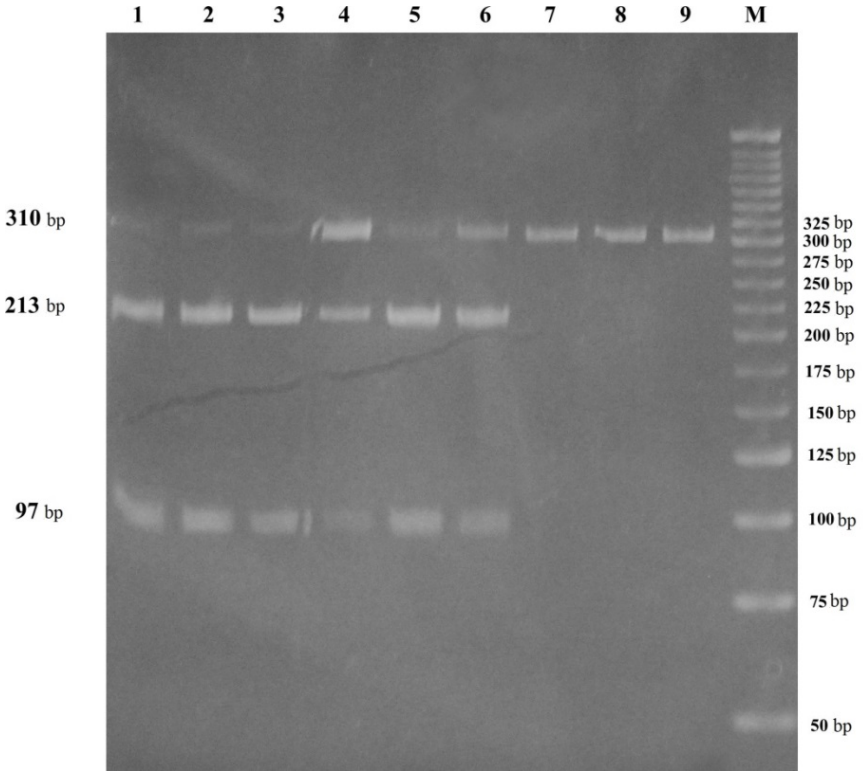
When processing the PCR products with restriction enzymes, the following DNA fragments were obtained: Thr/Thr-181, 101, 44, 30 bp.; Arg/Arg – 310 bp., Arg/Lys -310, 213, 97 bp, Lys/Lys - 213, 97 bp; Asp/Asp - 207,126 bp; Arg/Arg-501,86,71 bp.

Results and discussion. In order to observe *MYOC* and *NTF4* polymorphism by PCR-RFLP methods 85 patients suffering from glaucoma and 100 glaucoma-free individuals as a control group were included in genetic case-control studies. The genotype distribution based on SNPs are shown in figures 1 and 2.



M – DNA Ladder 25 bp (*ThermoFisher Scientific*, USA), 1-11 – CC genotype.

Figure 1 – R76K SNP genotyping results by PCR-RFLP method



M – DNA Ladder 25 bp (*ThermoFisher Scientific*, USA),
1, 2, 3, 5 – GG genotype, 4, 6 – GA genotype, 7, 8, 9 – AA genotype.

Figure 2 – Results of R76K SNP polymorphism by PCR-RFLP method

The frequencies of R76K polymorphisms in glaucoma patients and healthy control group are tabulated in table 2.

Table 2 – Genotype frequencies of the studied R76K G>A polymorphism in population

SNP	Genotypes	Patients group n=85	Patients group n=100	x^2	OR	CL 95%	P
R76K G>A	GG	0.682	0.970	26.59	0.07	0.02 – 0.23	3.0E-7
	GA	0.259	0.030		11.29	3.24 –39.30	
	AA	0.059	0.000		13.73	0.75 -252.07	

It this study R76K SNP has been detected in 27 glaucoma patients (22-Arg/Lys, 5-Lys/Lys) and in 3 individuals (3-Arg/Lys) from the control group.

R76K polymorphism is nucleotide change resulted in *G* being replaced by *A* (c.227G>A) in exon1 of *MYOC/TIGR* gene predicting amino acid change (substitution of Arg by Lys). The research carried out in Germany shows that R76K SNP was detected in 40 out of 112 glaucoma patients and in 3 patients there was identified Lys76Lys mutation at a polymorphic level [17].

According to the data, the variation T353I c.1058C>T in exon3 of *MYOC* gene can be one of the main reasons for developing POAG with increased intraocular pressure [15]. The reported findings on glaucoma genetics in Chinese Han population add to a growing body of evidence supporting that hypothesis. However, the genomic analyses of populations in Caucasus and Africa revealed that there is no genetic association of mutation T353I with glaucoma. Thus, genetic variations of *MYOC* gene can be varied among different ethnicity [16]. D208E mutations of *MYOC/TIGR* gene are causes of amino acid changes (Asp to Glu) due to the 624-cytosine nucleotide in exon2 of this gene is substituted by guanine. F. Mabuchi et al. reports that Asp208Glu mutations have been found in 4 hypertensive glaucoma patients and 3 POAG patients, at the same time it was revealed in 1 individual from the control group in Japan [18]. In addition, Japanese researchers investigated the distribution of Asp208Glu polymorphism among 99 glaucoma patients and their families also, in which Asp208Glu SNP has been indicated in one of the patients' mother and the researchers considered Asp208Glu polymorphism as an occasional neutral change with no effect on the gene's output [26]. Consequently, genetic variations of *MYOC* gene can be varied among different ethnicity.

The studies were carried out in China showed that R206W polymorphism in *NTF4* gene is a rare example of mutation. This SNP was indicated only in one of the 174 patients [27]. The research held by Pasutto found that named SNP was indicated in 4 glaucoma patients out of 399 from the experimental group and in none of the controls [28]. Abundant studies lead us to conclude that R206W polymorphism is a very rare SNP. Indeed, as a result of our study R206W polymorphism has been detected in neither case belong to the test group nor the control group.

By the frequency of T353I, D208E and R206W polymorphisms in *MYOC* gene and in *NTF4* respectively, there is no statistically significant difference between patients with POAG and individuals from control group. The available data point to the low frequency of above-mentioned polymorphisms. Our study has revealed that R76K is the most frequent polymorphism in Kazakhstan population. According to literary sources, the frequency of R76K SNP in Asia's population is higher than in Europe. The results of our research suggest that T353I, D208E and R206W polymorphisms in Kazakhstan population are the matter of neutral SNPs and could not be determined as a genetic reason of POAG progression, whereas R76K SNP has a strong association with POAG form of glaucoma.

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ҚАЗАҚСТАНДА ГЛАУКОМАМЕН АУЫРАТЫН НАУҚАСТАРДА МИОЦИЛИН (*MYOC)* ЖӘНЕ НЕЙРОТРОФИН 4 (*NTF4)* ГЕНДЕРІН ТАЛДАУ

Аннотация. Қазіргі таңда біріншілік ашық бұрышты глаукома офтальмология саласындағы медициналық-әлеуметтік мәнге ие басым бағыттардың бірі болып саналады. Глаукома 21-50% жағдайда генетикалық негізделген, глаукомамен ауыратын науқастардың ұрпақтарында осы аурудың даму қаупі 10 есеге жоғары болатындығы анықталған. Біріншілік ашық бұрышты глаукома ауруын тудыратын мутациялар 57кДа миоцилин белогын кодтайтын *MYOC/TIGR* және 28 кДа димерлі полипептидті кодтайтын *NTF4* гендерінде анықталған. Бұл жұмыста Қазақстан популяциясында *MYOC/TIGR* және *NTF4* гендеріндегі мутациялардың кездесу жиілігі анықтау қарастырылған. Зерттеуге біріншілік ашық бұрышты глаукомамен ауыратын 85 науқас және бақылау ретінде 100 сау адамдардан жиналған қан үлгілері қолданылды. Зерттеу нәтижесінде *MYOC/TIGR* геніндегі T353I, D208E және *NTF4* геніндегі R206W мутациялары мен глаукома ауруының дамуы арасында байланыстың болмайтындығы, ал *MYOC/TIGR* геніндегі R76K SNP мутациясының аталған аурудың дамуына әсер ететіндігі анықталды.

Түйін сөздер: БАБГ, полиморфизм, *MYOC/TIGR*, *NTF4*.

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АНАЛИЗ ГЕНОВ МИОЦИЛИНА (*MYOC*) И НЕЙРОТРОФИН 4 (*NTF4*) У БОЛЬНЫХ ГЛАУКОМОЙ В КАЗАХСТАНСКОЙ ПОПУЛЯЦИИ

Аннотация. Первичная открытая глаукома, являющаяся наиболее распространенной формой глаукомы, имеет большое социально-медицинское значение и является основным направлением научных исследований в области офтальмологии. В 21-50% случаев заболевание обуславливается генетически, а у потомков людей, болевших глаукомой, риск этой патологии в 10 раз выше. Мутации, вызывающие первичную открытую глаукому, были идентифицированы в гене *MYOC/TIGR*, который кодирует белок, известный как миоцилин (57 кДа), и в гене NTF4, который кодирует димерный пептид (28kD). В данном исследовании мы изучали частоту мутаций в генах *MYOC/TIGR* и NTF4 среди населения Казахстана. Исследование проводилось с участием 85 пациентов с первичной глаукомой и 100 человек в качестве контрольной группы. Результаты наших исследований показывают, что полиморфизмы T353I, D208E в гене *MYOC/TIGR* и R206W в гене *NTF4* среди населения Казахстана не влияют на прогрессию ПОУГ, тогда как R76K SNP в генах *MYOC/TIGR* может быть генетическим фактором, который влияет на развитие глаукомы типа ПОУГ.

Ключевые слова: ПОУГ, полиморфизм, *MYOC/TIGR*, *NTF4*.

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STATE OF NATURAL FEEDING BASE OF FISH-BREEDING PONDS BY BREEDING OF FINGERLINGSOF THE PIKEPERCH

Abstract. Thepurpose of this work was a determination of the level and the dynamic ofdevelopment the phytoplankton and zooplanktonin experimental ponds which were used for breeding the fingerlingsof pikeperch in polyculture with common carp and plant-eating carps. An importance of studying the level and the dynamic of development the phytoplankton and zooplankton in ponds which were used for breeding the fingerlingsof pikeperch in polyculture with common carp and plant-eating carps is substantiated. The methods of studying the level and the dynamic ofdevelopment the phytoplankton and zooplanktonin experimental ponds are presented. The level of phytoplankton and zooplanktonin experimental ponds in which was hold breeding the one-years of pikeperch from the fingerlings,is shown. The composition of species, the level of development of phytoplankton and zooplanktonin experimental ponds in different years of researchesis shown. The dynamics of the level of development of phyto-plankton and zooplanktonin some months of the determined year of holding the researchesis shown. The fact that holding of measures according to the stimulation of development the natural feeding base which are the using the fertilizers etc. is influencing for increasing the biomass of organisms of zooplankton especially in the end of fish-breeding season, is shown. The conclusions in which presented the dynamic of development of phytoplankton and zooplankton of experimental ponds in different months of the year are given. The importance of hydrobiological researches by breeding the one-years of pikeperch in fish-breeding ponds is shown. The recommendations according to the results of researches the dynamic and biomass of phytoplankton and zooplankton are given. The period of fish-breeding season in which holding the works according to the maintenance of biomass of phytoplankton and zooplankton in fish-breeding ponds which are using for the breeding of one-years of pikeperch is recommended.

Keywords: phytoplankton, zooplankton, dynamicof development, fish breeding in ponds, one-years, pike-perch.

Introduction. Currently, with the renovation of aquaculture as an industry in Kazakhstan, one of the ways to develop fish-breeding enterprises, in particular pond farms, is the development of fish seeds production for the needs of aquaculture with the aim of stocking natural reservoirs for the reproduction of commercial populations, and also for growing commodity fish production.

One of the technological parameters in pond fish farming is the biomass of phyto, zooplankton and macrozoobenthos by the cultivation of fish during the seasonof cultivation, on the basis of which it is possible to obtain indirect information on the pond's nutrition, which allows to optimally plan the measures to increase productivity, usually, up to a certain limit, with no additional expenses of feed. This increases the economic efficiency of pond farming.

One of the new objects of aquaculture of Kazakhstan is the pikeperch. The high taste qualities of this fish make it possible to export a significant part of the fillets to Europe. At the same time, the repro-duction of stocks of pikeperch in reservoirs of the country is particular urgency. Studies of LLP "Kazakh Science Research Institute of Fisheries" have shown the possibility of cultivation the pikeperch in carp ponds in polyculture with two-years of carp and the herbivorous fish.

The state of the natural forage of ponds during the cultivation of pike perch is the great scientific and practical interest.

Material and methods

According to materials obtained by the farmers of Hungary, phytoplankton and zooplankton are most great important for the fingerlings of pikeperch. The development of phytoplankton contributes to the increase of the biomass of the smallest zooplankton (Rotatoria), and then of largest forms of forage planktonic crustaceans (Cladocera, Copepoda) [1].

The materials for research were the compositionof species, abundance and biomass of phytoplankton and zooplankton in experimental ponds used for growing the pikeperch in a polyculture with two-years ofcommon carp, grass carp and silver carp.

The research of the state of phytoplankton and zooplankton of experimental ponds was carried out using standard methods adopted in hydrobiological researches [2-5].

The state of the natural food supply of experimental ponds was compared with the growth of pikeperch and the level of fish productivity [1, 6-20].

Results and discussion

Phytoplankton. According to the results of processing of samples taken in experimental ponds in the spring-and-summer period of 2013-2014, 52 species of algae belonging to 6 divisions, among them: Cyanophyta - 10, diatoms - 23, green - 12, pyrophytic - 3, euglenic - 3, golden - 1 species. The following species of algae were dominant: *N.gregaria, Achnanthes sp., C. vulgaris, P. achromaticum, C. meneghiniana, E. cordata, P. achromaticum, Trachelomonas sp*. The minimum amount of algae occurs in ponds was in May. In summer, in June and July phytoplankton in the ponds developed more intensively.

Dynamics of biomassof phytoplankton in experimental ponds in the 2013-2014 seasons is presented in table 1.

Table 1 – Dynamics of quantitative development (biomass, g/m^3) of phytoplankton in experimental ponds in 2013-2014

Algae	2013				2014			
	May	June	July	August	May	June	July	August
Green algae	–	0,040	0,250	0,020	0,085	0,015	0,020	0,030
Cyanophyta	0,020	0,155	0,225	0,001	0,008	0,045	0,039	0,055
Diatomaceous	0,065	0,955	2,00	0,855	0,050	0,900	0,645	0,400
Euglenic	–	0,150	0,105	0,075	0,040	0,950	0,040	0,200
Pyrophytic	0,015	0,215	0,475	0,100	0,200	0,050	0,070	0,400
Golden	–	–	–	–	0,010	–	–	–
Average	0,100	1,515	3,055	1,051	0,393	1,960	0,814	1,085

In spring the phytoplankton poorly developed in two seasons. According to the terms of the phytoplankton biomass in May the ponds were classified as low-grade nutrition β-oligotrophic type.

According to the terms of biomass of phytoplankton in June ponds can be considered as reservoirs of a moderate class of nutrition α-mesotrophic type.

In July 2013, the ponds in terms of the biomass of algae corresponded like the medium-grade nutrition β-mesotrophic type.In July 2014, the ponds to the reservoir of the moderate grade nutrition were likeβ-mesotrophic type. In August ponds by the size of phytoplankton biomass could be considered as reservoirs of a moderate class nutrition α-mesotrophic type [5].

According to the "scale of trophy" byKitaevS.P.ponds in June in terms of phytoplankton biomass corresponded like the middle class of nutritious, the β-mesotrophic type. In July and August nutrition began to correspond to the moderate, and the type of reservoir wasα-mesotrophic [5].

The basis of phytoplankton biomass in June was like euglene algae (54.8%), in July was mostlyof diatoms (56.9%), and in August pyrophyticalgae (55.5%). The taxonomic list of algae selected in experimental ponds in 2015 numbered 62 species of algae belonging to 5 divisions. Among them: Cyanophyta - 14, diatoms - 22, green - 20, pyrophytic - 4, euglenic - 2. The dominant phytoplankton complex is represented by the following species of algae: *N. gregaria, C. meneghiniana, A. ovalis, C. vulgaris,*

C. undulatum ,Trachelomona ssp., E. cordata. The smallest number of algal species was recorded in May-and-June and varied from 4 to 9 taxa. The taxonomic composition of algae varied from 13 to 21 taxa. In the spring the phytoplankton was developed poorly, the biomass was low and varied from 0.265 to 0.691 g/m^3.

The composition of dominant species varied during the summer period. So, in June pyrophyticalgae (56%) dominated in the reservoir, in July dominated the diatoms (34.4%), and in August – the blue-green algae (42%).

In June-July 2015, the biomass of phytoplankton (0.365-2.665 g/m^3) corresponded to a moderate class of nutritious, α-mesotrophic type. In June the basis of biomass was pyrophyte algae (71.4%), and in July - diatoms (35.7%). In August the level of water supply increased to the middle class, and the type of reservoir to β - mesotrophic type (when a number of biomass is 0.360-3.015 g/m^3). The fundament of phytoplankton at this time was diatoms (62%).

Zooplankton. According to the results of the hydrobiological survey in the period April-August of2013, the zooplankton of the experimental ponds is represented by 55 taxa from three main groups, where 26 taxa are rotifers, 16 cladoceraand 13 copepods. In addition to zooplankton organisms, a large number of facultative zooplankers have been found in the samples: ostracods, insect larvae, worms, hydra.

The greatest taxonomic diversity was found in the pond #3 - 43 taxa, 21 - rotifers, 12 - cladocera and 10 - copepods. In the pond #4, 31-16-9-6 taxa, respectively, were identified. The main background of the zooplankton community in both ponds was (33-45% of the occurrence for the spring-summer period) *S. pectinata, A. sieboldi, L. ungulate, E. d. dilatata, C. laticaudata, S. mucronata, Ch. sphaericus and M.leuckarti.* In the pond No.3 they were supplemented with *E. pyriformis, Br. q. melheni, D. macro-phthalma, P. Trigonellus, in pond No. 4 - C. reticulate, N. incongruens.*

In pond #4, the basis of abundance and biomass were the cladocera - 75.4% according to thenumber of specimens and 84.3% according to the biomass. Production indicators per m^3 in the pond began to grow from June to mid August, did not fall below 65.0 thousand individuals and 1.5 gper 1 m^3, reaching its peak in early July - 263.7 individuals and more than 9.0 grams per 1 m3, due to the mass development of the cladocera crustaceans of the genus Ceriodaphnia, whose share in the total indices was from 30 to 94 %.

In the pond #3 the basis of abundance was copepods - 41.6%, biomass in the pond formed a Cladocera - 48.2%. During the season, the largest production indicators were revealed in May, due to the massive development of large representatives of the Cladocera crustaceans D. galeata, S. vetulus, S. mucronata, and in the mid-period July-August, due to the development of large predatory rotifers of the genus *Asplanchna*, C laticaudata and younger age stages of copepods (nauplii and copepods).

Hydrobiological analysis of the natural forage reserve of experimental ponds in the 2012-2014 seasons showed that zooplankton is represented by 55 taxa from three main groups, where 26 taxa are rotifers, 16 cladoceraand 13 copepods. In addition to zooplankton, a large number of facultative zooplankers - ostracods, insect larvae, worms, hydra, were found in the samples.

The determining role in the plankton of all the ponds during the vegetation seasons belonged to the Cladocera crustaceans. Their stable dominance in the total mass of zooplankton in the experimental ponds was noted.

Analyzing dynamics of development ofzooplankton during the season in 2012-2014, in experimental ponds it is clear that after flooding of experimental ponds, the quantitative indices of zooplankter abundance and biomass were identically low and were within 19.04-39.76 thousand individuals/m^3 and 0.355-0.827 g/m^3, respectively.

The dynamics of quantitative indicators of zooplankton in experimental ponds in 2012-2014 is presented in table 2.

For increasing the level of the natural food supply in ponds, intensification measures were carried out, which stimulated the development of hydrobionts. The organic fertilizers (manure of cattle) were brought at the rate of 2 t / ha; inorganic (ammonium nitrate at the rate of 20 kg/ha, superphosphate - 10 kg/ha); sheaves of the dried up aquatic plants (reed, cattail); culture of daphnia (1 l / ha); fodder yeast (1 kg/ha).

The results obtained after stimulation indicate a general tendency for the growth of quantitative parameters, which reach their maximum in 2012-2014 in the first decade of May (129.1-246.6 thousand individuals/m^3 and 3.817-5.46 g/m^3, respectively). These indicators characterize the experimental ponds during this period, as highly nutritious ponds [5].

Table 2 – Dynamics of number and biomass of zooplankton in experimental ponds in 2012-2014

Date	2012		2013		2014	
	thousand individuas / m^3	g/m^3	thousand individuas / m^3	g/m^3	thousand individuas / m^3	g/m^3
14.04.	25,7	0,827	39,760	0,640	19,04	0,355
28.04.	89,5	1,738	10,90	0,126	29,860	1,485
15.05.	129,1	3,817	246,60	4,441	136,70	5,460
31.05.	101,0	1,864	65,970	1,763	63,50	2,189
14.06.	112,1	4,367	93,450	2,579	55,040	2,009
29.06.	129,1	3,817	87,920	3,071	53,0	1,962
15.07	155,5	2,163	84,511	2,823	31,68	1,660
30.07.	165,0	2,792	98,176	2,740	10,87	0,10
15.08.	76,5	1,349	17,10	0,382	8,80	0,069

On the parameters of quantitative zooplankton development in 2012 and 2013 in general, the level ofnutritious of ponds washigh. In the season of 2014, the dynamics of zooplankters development characterizes the ponds as medium nutritious level [5]. Probably the decrease in the quantitative indicators of plankton is associated with the increase in the ponds of carp individuals. In 2014, the density of carp seeds in polyculture with pike perch was 1000 pcs / ha. As we know, the zooplankton in yearlings of carp is the main diet during the first half of fish-breeding season.

At the end of the season, the nutrition of all the ponds in 2012-2014 decreased. According to the classification of feed, all the ponds during this period corresponded to low nutritious level [5].

According to the results of hydrobiological researches in the spring-and-summer period of 2015, zooplankton in ponds of the Chilik farm was represented by 66 taxa from three main groups, where 32 taxa are rotifers, 16 cladoceraand 18 copepods. The main population of the zooplankton in both ponds were*A. girodi, A. sieboldi, A. brightwelli, L. bulabula, Br. c. amphiceros, D. macrophthalma, C. reticulata, C. quadrangula, M. brachiata, S. mucronata, D. crassa, Ch. sphaericus, A. americanus* and *M. leuckarti* (57-100% of the occurrence in the spring-summer period).

If we consider by months, the largest number of species in the ponds was found in June-July, 46-44 taxa, respectively, the smallest in August - 22 species. Quantitative development of zooplankton in ponds is presented in table 3.

The basis of abundance and biomass in pond #3 generally were Cladocera crustaceans - 74.5 and 87.0% respectively. Throughout the growing season they prevailed in the community, but in the beginning of June rotifers became the dominant group, where predatory rotifers of the genus Asplanchna (42.7% abundance and 72.9% in biomass). During the season the highest production indicators were revealed in the period from mid of June to mid of July, with a peak in mid of July (14,929-12,369-21,369 g/m^3), when the biological productivity of the pond reached a very high great eutrophic-hypertrophic type due to a massive development of the cladocera crustaceans of the genus *Ceriodaphnia* (81.3-92.5-95% of the total biomass). In May, a large number of facultative plankton organisms were noted in the samples, where ostracods were most important, their share in the total biomass was about 26.0%.

The basis of abundance and biomass in the pond #4 was Cladocera crustaceans - 92.1 and 96.8% respectively. The highest biomass indicators were detected in June - 22,121-22,627 g/m^3 and in the middle of July (32,528 g/m^3), where the basis was composed by Cladocera of the genus *Ceriodaphnia* (97.4-98.0-97.1% of the total biomass).

The basis of abundance and biomass in the pond #1 were Cladocera crustaceans - 74.5 and 87.0%, respectively. During the vegetation period the maximum production parameters were revealed during the period - the end of June to the end of July, with a peak in the middle of July (3.156-5.830-3.220 g/m^3), due to the mass development of the Cladocera crustaceans of the genus *Ceriodaphnia* and *Moina* (46.4-53, 9-51.8% of the total biomass).

Table 3 – Quantitative development of zooplankton in experimental ponds in 2015,
N – number, thousand specimens / m^3, B – biomass, g/m^3

Sampling day	Rotifers		Cladocera		Copepoda		Total	
	N	B	N	B	N	B	N	B
Pond #3								
20.05	11,90	0,116	76,0	5,027	30,0	0,663	117,90	5,806
05.06	235,90	5,337	54,80	1,268	32,20	0,638	322,90	7,243
20.06	10,30	0,128	430,0	14,401	59,50	0,413	499,80	14,942
05.07	15,0	0,082	331,0	11,915	44,30	0,249	390,30	12,246
20.07	20,60	0,091	500,0	20,757	38,0	0,511	558,60	21,369
05.08	1,20	0,021	96,0	3,407	10,30	0,263	107,50	3,691
Average index	49,150	0,963	247,967	9,463	35,717	0,456	332,834	10,882
Pond #4								
20.05	1,80	0,002	95,0	7,416	21,20	0,105	118,0	7,523
05.06	24,0	0,045	875,20	21,899	33,60	0,177	932,80	22,121
20.06	8,0	0,027	644,0	22,290	48,30	0,310	700,30	22,627
05.07	6,0	0,126	128,90	4,959	9,30	0,053	144,20	5,138
20.07	3,0	0,007	879,80	32,133	32,60	0,388	915,40	32,528
05.08	20,0	0,10	195,0	5,672	33,80	1,825	248,80	7,597
Average index	10,467	0,051	469,650	15,728	29,80	0,476	509,917	16,255
Pond # 1								
12.06	15,20	0,070	23,50	0,711	6,20	0,030	44,90	0,811
27.06	22,60	1,052	39,80	1,545	25,20	0,559	87,60	3,156
12.07	59,0	1,619	80,40	3,301	45,20	0,910	184,60	5,830
27.07	23,30	0,388	56,30	1,856	22,0	0,976	101,60	3,220
12.08	9,20	0,216	33,0	1,257	32,60	0,197	74,80	1,670
Average index	25,860	0,669	46,60	1,734	26,240	0,534	98,70	2,937
Pond # 5								
12.06	2,40	0,005	213,60	5,306	30,60	0,350	246,60	5,661
27.06	42,60	1,514	48,40	1,80	14,40	0,609	105,40	3,923
12.07	34,0	0,649	69,60	2,548	39,90	1,190	143,50	4,387
27.07	64,0	2,325	122,0	4,769	52,0	0,995	238,0	8,089
12.08	12,0	0,245	87,0	3,726	52,0	1,139	151,0	5,110
Average index	31,0	0,947	108,12	3,630	37,780	0,857	176,90	5,434

The basis of quantitative parameters in the pond #5 during the study period were Cladocera crustaceans - 61.1% by the number of specimens and 66.8% by biomass. The biomass of zooplankton during the season varied from 3.923 g/m^3 in late June to 8.089 g/m^3 at the end of July, which corresponds to high class of food level with the eutrophic type. Among the species dominated were the genus *Ceriodaphnia* - 42,8%, rotifers of the genus *Asplanchna* - 16,2%, *M.brachiata* - 10,9%.

During the researchingperiod,Cladocera crustaceans dominated in all the ponds, among them the crustaceans of the genus *Ceriodaphnia*, whose proportion in the total mass reached 98%.

Among studied ponds, #3 and #4 were the most productive in terms of zooplankton, where the parameters ranged from a medium-class mesotrophic class to a very high-class hypertrophic. The

productivity of zooplankton in ponds No. 1 and #5 was somewhat lower, the parameters varied from a low-great oligotrophic class to a highly eutrophic type [5].

The need for increasing the level of zooplankton development in ponds, where pikeperch is grown, is also indicated by Russian and Belarusian scientists [6-9].

Usually, an increase in the level of development of zooplankton in the first half of the hatchery season is the key to improving the fish productivity of perch pike. In the second half of the hatchery season, the zooplankton plays a secondary role as food. The main prey in this period of time is fish juveniles [10-13].

As can be seen from the presented data, the greatest growth of phytoplankton biomass is observed in the period "May-June", the greatest level of zooplankton development occurs in July, then, in August the biomass of phytoplankton goes down.

Conclusions.

1) In general, based on the results of hydrobiological studies of natural forage base of experimental ponds it is clear that the level of biomass of phytoplankton and zooplankton was optimal for pike perch cultivation.

2) At the beginning of the fish-breeding season, immediately after filling the ponds, the level of phyto- and zooplankton development is usually low. Taking into account the early stocking of pikeperch in ponds, which grows in the conditions of pond farms in the South Kazakhstan at the beginning of May,it is necessary to organize earlier filling of the ponds and carry out a set of measures to increase the level of phyto- and zooplankton development.

3) It is necessary to support the development of phyto- and zooplankton in the first half of the hatchery season. Further, the level of development of the natural forage of ponds is quite high.

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СОСТОЯНИЕ ЕСТЕСТВЕННОЙ КОРМОВОЙ БАЗЫ ПРУДОВ ПРИ ВЫРАЩИВАНИИ СЕГОЛЕТОК СУДАКА

Аннотация. Целью работы было определение уровня и динамики развития фитопланктона и зоопланктона в экспериментальных прудах, занятых под выращивание сеголеток судака в поликультуре с карпом и растительноядными рыбами. Обоснована важность изучения уровня развития фито- и зоопланктона в прудах, занятых под выращивание сеголеток судака в поликультуре с карпом и растительноядными рыбами. Представлены методики изучения уровня и динамики развития фитопланктона и зоопланктона в экспериментальных прудах. Показан уровень развития фито- и зоопланктона в экспериментальных прудах, в которых проводилось выращивание сеголеток судака от подрощенной молоди. Показаны видовой состав, уровень развития организмов фито- и зоопланктона экспериментальных прудов в разные года проведения исследований. Показана динамика уровня развития фито- и зоопланктона в отдельные месяцы определенного года проведения исследований. Показано, что проведение мероприятий по стимуляции развития естественной кормовой базы (внесение удобрений и др.) оказывает непосредственное влияние на увеличение биомассы организмов зоопланктона, особенно к концу сезона эксплуатации рыбоводных прудов. Даны выводы, в которых представлена динамика развития фито- и зоопланктона экспериментальных прудов по месяцам года, показано значение гидробиологических исследований при выращивании сеголеток судака в прудах. По результатам исследований динамики и биомассы фито- и зоопланктона даны рекомендации, в какой период рыбоводного сезона наиболее целесообразно проводить работы по поддержанию биомассы фито- и зоопланктона в рыбоводных прудах, занятых под выращивание сеголеток судака.

Ключевые слова: фитопланктон, зоопланктон, динамика развития, прудовое рыбоводство, сеголетки, судак.

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ОСЫ ЖАДЫҚ КӨКСЕРКЕ БАЛЫҒЫН ӨСІРУДЕГІ ТОҒАНДАРДЫҢ ҚОРЕКТІК БАЗАСЫНЫҢ ТАБИҒИ КҮЙІ

Аннотация. Жұмыстың мақсаты тұқы мен шөппен қоректенетін балықтармен поликультура жағдайында осы жаздық көксерке балықтары өсірілген тәжірибелік тоғандардағы фитопланктон мен зоопланктонның даму динамикасы мен деңгейін анықтау болды. Мақалада осы жаздық көксерке балықтарын тұқы мен шөппен қоректенетін балықтармен бірге өсірген кездегі тоғандардың фито- және зоопланктон даму деңгейін зерттеу маңыдылығы көрсетілген. Тәжірибелік тоғандардағы фитопланктон мен зоопланктон даму динамикасы мен деңгейін зерттеудің әдістемелері келтірілген. Фито- және зоопланктон даму динамикасы зерттелген тәжірибелік тоғандарда осы жадық көксерке балықтары өскелең шабақ кезеңінен бастап өсірілген. Тәжірибелік тоғандардағы бірнеше жылдық зерттеу жұмыстарын жүргізу барысындағы фито- және зоопланктон ағзаларының даму деңгейі мен түрлік құрамы көрсетілген. Белгілі бір жылдарда зерттеу жұмыстарын жүргізген кезеңдердегі, жекелеген айлардағы фито- және зоопланктон даму деңгейінің динамикасы көрсетілген. Табиғи қоректік базаны қолдан арттыру үшін жасалатын іш шаралар (тыңайтқыштарды салу және т.б.) тікелей зоопланктон биомассасын арттыруға оң әсер ететіндіктері анықталған, әсіресе балық өсіретін тоғандарды пайдалану маусымының соңына қарай. Қортындыда тәжірибелік тоғандардағы фито- және зоопланктон даму динамикасы айма-ай көрсетілген, тоғандарда осы жаздық көксерке балығын өсірудегі гидробиологиялық зерттеу жұмыстарының маңыздылығы баяндалған. Фито- және зоопланктон динамикасы мен биомассасын зерттеу нәтижелері бойынша балықты қолдан өсіру маусымында осы жаздық көксерке балығын өсіру барысында фито- және зоопланктон биомассасын біркелкі әрі тұрақты етіп ұстап отыру үшін қажетті ұсыныстар берілген.

Түйін сөздер: фитопланктон, зоопланктон, даму динамикасы, тоған балық шаруашылығы, осы жадық балықтар, көксерке.

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STUDYING THE I/D *ACE* AND R/X *ACTN* ASSOCIATIONS OF POLYMORPHISM WITH THE LEVEL OF PHYSICAL PREPATIBILITY OF KAZAKHSTAN'S FOOTBALLERS FOR DEVELOPMENT OF GENETIC AND PHYSIOLOGICAL METHODS OF SPORTS SELECTION

Abstract. In this research work on the basis of molecular genetic analysis and the study of basic physiological parameters, a correlation analysis was made between the genotypes of I/D *ACE* and R/X *ACTN* polymorphisms and the level of physical fitness of Kazakhstan professional football players. The obtained results testify to the prospects of using these polymorphisms for the development of genetic methods of sports selection.

Keywords: sports selection, molecular genetic markers, gene polymorphisms, physiological parameters, anthropometry, volumoscopy, bioimpedanceometry (Tanita), chronometry, lactometry.

Sports activities currently impose athletes high demands. In modern sports the great importance is for technical and tactical readiness of the athlete, psychological readiness to overcome large sports strain. Scientifically grounded search and selection of talented sports youth is a trend of the time and the main task of sports selection, therefore the role of the trainer is very important, he is capable to reveal sports opportunities of each individual and correctly organize training process. Important prerequisites that must be taken into account during the coach sports selection: biological factors, especially morphology (physique), the type of nervous activity, the level of aerobic and anaerobic capabilities, the ratio of fast and slow muscle fibers. The relevance of the problem of sports selection increases with renewed vigor, as the available methods of sports selection are not effective enough and do not meet all modern requirements. An important condition for the development of modern sports is a scientifically based search for talented youth, which can handle large sports strain and high rates of sports improvement.

Thus, sports selection is a system of organizational and methodical actions including pedagogical, psychological, sociological and medico-biological methods of research on the basis of which abilities of children, teenagers and young men for specialization in a certain sport or group of different sports are revealed [1].

Throughout the world, at the initial stage, the determining markers of predisposition to sports activities were: blood groups, body type, dermatoglyphics, the composition of muscle fibers, the type of sensorimotor reactions and other phenotypic features. According to the history of sports selection, earlier success of the athlete judged on the basis of studying of morphometric and physiometric parameters of the individual [2]. Today genetics of physical activity develops rapidly, it includes sports genetics and some areas of anthropogenetics and medical genetics. Molecular genetic studies allow to achieve the highest success in sports, improve and help sports-orientation selection of young athletes, to optimize the level

and intensity of training strain develop for each athlete a special diet, because success in any sphere of human activity, including sports, depends on the genotype by 75-80%, and only 15-20% of success is due to education, training level and all other environmental factors [3].

Expression of interest and the future success of the individual in a particular sport, depends largely on the correct choice of sports specialization, in accordance with genetic factors possessed by the individual. The most important thing is that after the birth of a child, it is possible to predict and diagnose its future abilities and strong qualities, determine its future potential. For a sports-oriented selection of young athletes, despite the long experience of the trainers and teachers very often are wrong, incorrect predictions, subsequently, the athlete does not achieve considerable results in this sport, and it increases the risk of genetically - determined diseases. Thus, sports genetics is a much-needed area of research, on the basis of sports genetics will decrease the number of erroneous predictions, under the action of which many athletes are faced with a serious sports injury, which ultimately can even lead to death [4].

Hereditary information has a great impact on the morphological and functional characteristics and physical qualities of a person. The study of the degree of inheritance of various physiological parameters of the human body, shows the variability of the influence of genetics on physiology and the more pronounced hereditary effects on the signs of the body, the greater their account should be in sports selection. The greatest hereditary conditionality is revealed for morphological indicators of an organism, smaller for physiological parameters and the smallest for psychological parameters.

The most significant influence of inheritance on the following morphological features: longitudinal body size, volume size, body composition. The value of the coefficient of inheritance is the highest for bone tissue, less for muscle and the lowest for fat.

For physiological indicators revealed significant genetic conditionality, including most of the metabolic characteristics of the body, aerobic and anaerobic capabilities, the ratio of fast and slow fibers in the muscles, the volume and size of the heart, the characteristics of ECG, systolic and minute volume of blood at rest, heart rate during exercise, blood pressure, lung capacity and vital signs, the frequency and depth of breathing, minute volume of breathing, blood cholesterol, ESR and others.

The most psychological, psycho-physiological, sensor-motoric indicators placed under the expressed genetic control: rate of information processing, IQ coefficient, temperament, motor and sensor functional asymmetry and others. According to the results of scientific-research works, the fast movements that require specific rate of nervous system are affected by genetic control, high lability and nervous processes mobility, also development of organism’s anaerobic abilities and the presence of fast fibers in skeleton muscles. Thus, the most trainable physical features are agility and general endurance, the less trainable – rapidity and flexibility.

The knowledge of hereditary influence level on morpho-functional traits of human and his physical features give possibility during sports selection to lean on indexes, which are under genetic control, i.e more perspective and less changeable during training [5].

On the example of a number of European and Asian countries, we can see that sports results largely depend on the achievements of sports genetics. In developed countries, where sports genetics have long been recognized, there are high sporting achievements. World leaders in this area are the United Kingdom, Australia, China, Germany. Unfortunately, sports genetics is just beginning to develop in Kazakhstan. The use of ready-made commercial panels of genetic markers is impractical without studying the genotypic characteristics of domestic athletes, since sporting opportunities are determined by the interaction of the genotype with the ethnic background, specific living conditions (geography) and lifestyle. Therefore, to begin with, it is necessary to develop a panel of genetic markers applicable to the analysis of domestic athletes by testing the association of candidate genes with existing sports achievements and then become a real scientifically-based preparation of elite athletes for international competitions.

Kazakhstan's entry into the international sports arena and tough competition in all sports require new approaches to the development of physical culture and sports of the country. As foreign practice shows, the growth of sports achievements in Kazakhstan will not be possible without careful research and use of scientific developments, primarily sports genetics.

In accordance with the above, this study examined the association of polymorphisms I/D *ACE* and R/X *ACTN* with the level of physical fitness of football players in Kazakhstan for further development of educational and methodological approaches to determining the predisposition of students and schoolchildren to various sports.

Materials and methods

The work was performed on the basis of the laboratory of Molecular genetics RSE "Institute of General Genetics and Cytology" MES RK (Almaty). To conduct the research, an agreement was reached with the "Institute of sport in KazAST" on the collection of biological samples for molecular-genetic and physiological study. Thus, an experienced group consisting of 23 high-level football players was formed. Participation in this research was voluntary, all participants were familiarized with the main objectives of the research, completed questionnaires and signed informed consent to participate in the study. For each studied the questionnaire was composed and subsequently each athlete was taken buccally scraping from the inside of the cheeks.

DNA isolation. DNA from buccal scrapings was isolated using a kit of reagents for DNA extraction from clinical material "AmpliPrep DNA-Sorb-B", according to the manufacturer's protocol. The quantity and quality of the isolated DNA were estimated by a spectrophotometer, horizontal electrophoresis in 1,4% agarose gel. DNA samples were stored at -20°C and -80°C.

PCR. To detect polymorphisms of *ACE* I/D and *ACTN* R/X, the PCR method was used. Amplification was carried out in 20 μl of the total volume of the mixture containing 50 ng of genomic DNA. 10 μl 2xPCR Master Mix (0.05 U/μl TaqDNA polymerases, reaction buffer, 4 mM $MgCl_2$,0.4 mM of each dNTP (Thermo Fisher Scientific, USA) and 5pM of each primer. The following optimal conditions were selected for PCR: initial denaturation of 3 min at 95°C followed by 35 amplification cycles at 95°C for 30 sec., 56°C for r577x *ACTN*; 60°C for 287I/D *ACE* - 30 sec., 72°C 1 min and a final cycle of 72°C 7 min. PCR products were analyzed in 8% polyacrylamide gel and 1.4% agarose gel followed by visualization in passing UV light. The variants of genotypes were determined by the size of the allele-specific fragments: 287I/D *ACE* 190 pb – 287D allele and 480 bp – 287I allele. For the study R577X *ACTN* has set the restriction and fragmentation at the sites: 86 bp, 97 bp, 108 bp, 205 bp. PCR-RFLP mixture includes the PCR-product, buffer Tango, restrictase Ddel and H_2O.

Methods of statistical processing of results. Significance level (*p*) was determined using Chi2 and student's t-test and Chi-square test for degrees of freedom = 1 using the Calculator software for statistical calculation in “case-control” studies (http://www.tapotili.ru) with the additional amendment of Yates, provided for small samples. Differences between groups were considered statistically insignificant at $p>0.05$. To calculate OR and CI 95% used online calculator Medical statistics [6] and Biometrics [7].

Results and their discussion

The results of molecular genetic analysis of polymorphisms in genes 287I/D *ACE* and R577X *ACTN* of Kazakhstan football players. Gene *ACE* (angiotensin-1 converting enzyme-ACE) mapped in locus 17q23.3. More than 100 allelic variants of this gene are known, of which the most important in relation to physical activity is I/D polymorphism. Much attention paid to the study of the influence of muscle activity on the physiological parameters of the body in connection with the various allelic variants of *ACE*. Thus, a high correlation was established between the increase in the mass of the left ventricle of the heart after endurance training with an increased level of *ACE* in the blood and the D/D genotype. The association of its strength with D allele of *ACE* gene was established in the force training of the thigh quadriceps muscle [8].

These data were later confirmed in the measurement of isometric and isokinetic strength of this muscle in carriers of the genotype D/D [9].

Genotype I/I is associated with low activity of *ACE* gene and increased athletic endurance, human predisposition to successful sports aimed at the development of endurance and resistance to hypoxia in high altitude conditions. Carriers of genotype I/I have the greatest endurance. Also genotype I/I is associated with a higher percentage of type 1 fibers (slow-cutting fibers), which are more effective at long-term physical activity than fast-cutting type 2 fibers. Carriers of genotype I/I have the greatest endurance. The probability of age-related macular degeneration (the main cause of vision loss in old age) is 4.5 times lower than in groups with the genotype D/D and I/D. This genotype in most cases prevails in the group of stayers. Genotype I/I is the most favorable for such sports as marathon running, long distance swimming, skiing, biathlon, mountaineering, football, rugby, basketball, sports, martial arts that require endurance [10, 11]. Genotype D/D, on the contrary, is associated with higher activity of *ACE* gene and

manifestation of speed, strength and coordination abilities of sportsmen. The level of angiotensin-converting enzyme in carriers of genotype D/D increased in 2 times compared with genotype I/I. People with genotype D/D have lower stamina and they are not recommended excessive exercise. Carriers of D/D genotype have a risk of developing a large number of pathologies, in particular, such as myocardial infarction, arterial hypertension, hypertrophic cardiomyopathy. The effectiveness of muscle training in carriers of genotype D/D in 2 times lower than in individuals with genotype I/I. There is also a high risk of developing nephropathy in patients with diabetes [12, 13].

People with a heterozygous variant of I/D genotype have both variants of the gene and are carriers of the complex variant of the genotype and, as a rule, have good endurance, strength and speed. However, due to the presence of D allele, individuals with heterozygous *ACE* gene are not recommended excessive prolonged physical activity [14].

The distribution of genotypes according to the studied polymorphism of *ACE* gene (I/I, I/D, D/D) among athletes is indicated in figyre 1. In the research group were detected homozygous genotype 287I/I *ACE* gene. The frequency of homozygous genotype 287D/D of gene *ACE* (speed-strength qualities, coordination capabilities) amounting to 30.4 %. The frequency of heterozygous variant 287I/D of *ACE* gene (endurance, strength, speed, the presence of type 1 muscle fibers – slowly decreasing) was 69.6 %.

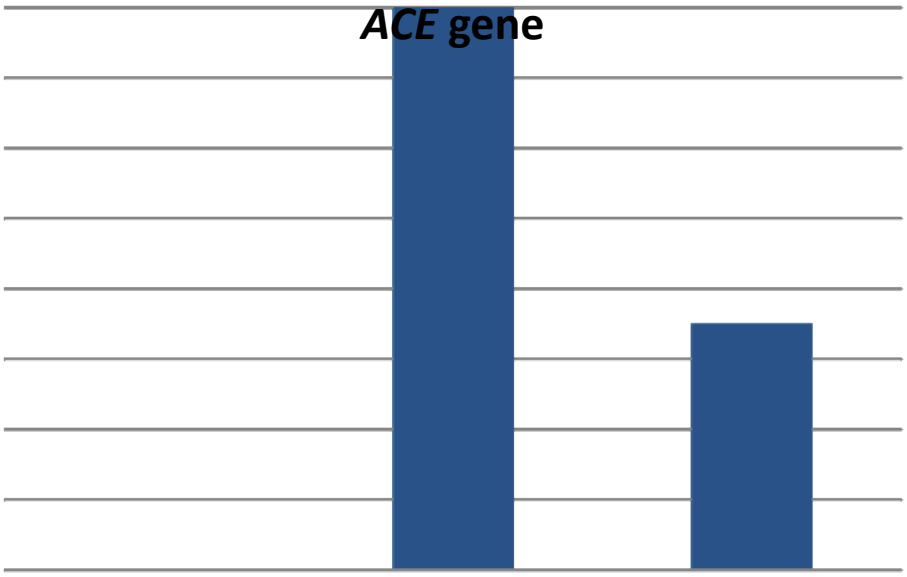

Figure 1 – Distribution of genotypes by the studied polymorphism of *ACE* gene (I/I, I/D, D/D) among the studied sportsmen

Our data confirmed the literature sources on the highest occurrence among athletes of complex heterozygous and the most favorable genotype I/D compared with homozygous variants [10, 11].

Thus, the analysis of the polymorphism association 287 I/D *ACE* in the group of young Kazakh professional athletes confirmed the trends noted by other scientific studies in the analysis of different populations.

ACTN3 gene is the first gene of the structural protein of skeletal muscles α-actinin-3, for which the connection with the manifestation of physical qualities of athletes, and genotypes *ACTN3* – one of the factors affecting the normal functioning of muscles. The product of the *ACTN3* gene is responsible for the synthesis of α-actin-3, which is the main component of the Z-lines of muscle sarcomeres, which determines the development of fast type II muscle fibers. The *ACTN3* gene is located in the long arm of 11 chromosomes (11q13-q14), consists of 20 exons and 19 introns.

Most people (6% in Africa, 19% in Europe, up to 25% in Asia) are homozygous for the X-allele polymorphism R577X of this gene [15].

As a result of replacement in 16 exons there is a stop codon, blocking the process of broadcasting iRNA, which leads to a deficiency of α-actinin-3. As a result of mutation, α-actin-3 is replaced by α-actin-

2, which leads to a decrease in the speed and power parameters of the athlete. R gene allele - presence of arginine – Arg amino acid at position 577 of *ACTN3* protein amino acid sequence. Gene X allele-designation of the terminal codon (stop codon)-Ter at the position 577 of the amino acid sequence of the protein *ACTN3*.

The R allele is more frequently diagnosed in athletes in sports that require explosive speed and power. Therefore, the carrier of R allele of *ACTN3* gene, the presence of alpha-actinin-3 protein in skeletal muscles, gives an advantage in the performance of speed-power strain, the energy supply of which is carried out by anaerobic mechanisms of ATP resynthesis. Carriers of genotype R/X is able to achieve good results at medium ranges and in sports that require a combination of speed, power and endurance. The X allele is prevalent in athletes who to achieve good results need endurance.

1. R/R (alpha-actinin-3 is present in sufficient quantities in muscle fibers);
2. R/X (alpha-actinin-3 is present in fewer muscle fibers than the RR genotype);
3. X/X (deficiency of alpha-actinin-3 in skeletal muscle).

Low frequency 577XX-genotype among athletes compared to the control indicates that in the process of sports selection has been the screening of athletes, whose muscle cells contained this myofibrillar protein.

Among skilled and highly skilled athletes discovered significant decrease in the percentage of XX-genotype in the group of speed-power kinds of sports and athletes involved in sports requiring endurance. Thus, functionally active α-actinin-3 (genotypes R/X and R/R) provides certain advantages for different types of human physical activity [16].

The distribution of genotypes according to the studied *ACTN* gene polymorphism (R/R, R/X, X/X) among Kazakhstan's athletes given in the figure 2. The frequency of homozygous genotype R/R of the *ACTN* gene was 21.7 %. The frequency of homozygous genotype X/X of *ACTN* gene was 30.4%. The frequency of occurrence of heterozygous variant R/X of *ACTN* gene (alpha-actinin – 3 is present in muscle fibers in smaller quantities, compared with the genotype R/R, rather good aerobic endurance, high speed-power abilities) was 47.9%.

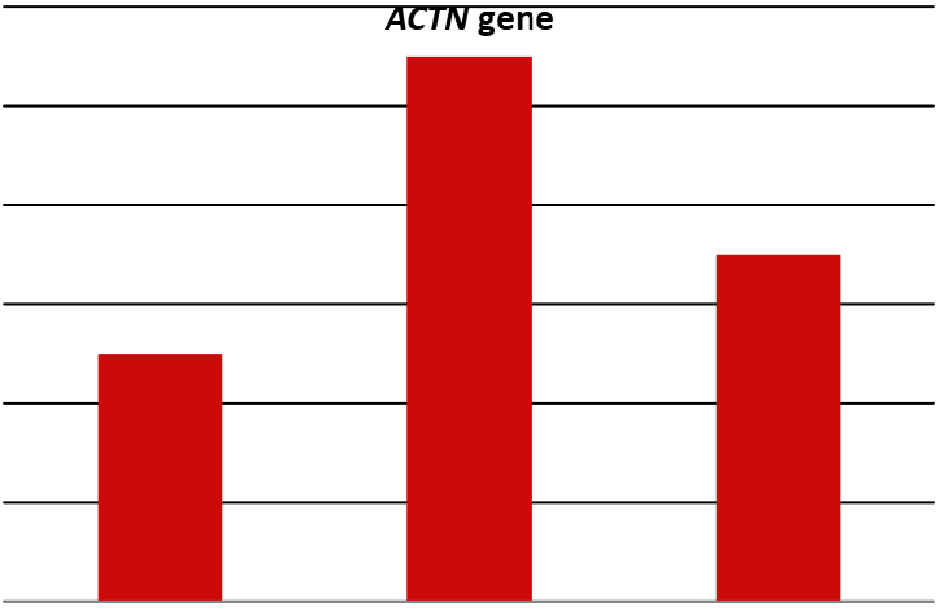

Figure 2 – Distribution of genotypes according to *ACTN3* (R/R, R/X, X/X) gene polymorphism among studied athletes

In the studied group of players, the most common are heterozygous variants of genes, which indicate the joint activity of favorable alleles. Due to the fact that the specific nature of football requires speed-strength qualities and a lot of endurance, that is the predominance of the heterozygous genotype in the studied group is expected and justified.

Association of genetic and physiological parameters of athletes. Most of the known performance parameters, both aerobic and anaerobic are important for the work of the players and achieve high results. So, on the one hand, the duration of the football match is more than 90 minutes, which indicates a high proportion of the aerobic mechanism, and on the other hand, short-term spurts are essential for the outcome of the match, in which the anaerobic source of energy is also crucial.

For the successful development of athletes training in terms of selection and projection is required 2 factors: genetic dispositions for adequate choice of sports specialization, style of competitive activity; multistage sampling at every stage of many years of training, taking into account genetically inherent in the athlete's speed of adaptation to specialized strain. High training, reducing the time of preparation of a highly qualified athlete, allows to perform biological (preservation of his health), social tasks (victory in competitions) and to achieve the high economic effect of the training process.

The examined athletes in terms of BMI body mass index were divided into two groups: control (model indicators included athletes with BMI < 20 at the age of 19-24 years, BMI=20-25 at the age of 25-34 years) and the case (players with BMI indicators that do not match the model).

Limits from 75 to 80 ml/kg were taken as a norm for such physiological parameter as a Life index (LI).

Also on the basis of the received data, the coefficient of speed endurance – CSE which indicators are shown in table 2 was calculated. The control group included athletes with CSE model indices 63% and more, other players joined the group – case. Therefore, there is a need to strengthen measures for the development of high-speed qualities of football players.

On such an important physiological parameter, such as lung capacity, the control group included athletes with LC=5100 and above, with LC to 5100 ml – group case.

On muscular weight, the athletes who were included in group – control had muscular weight 36 and above (good data), and in group a case < 36.

Regarding lactate, one of the main and indicative physiological indicators, measurements were made after 1 min., 3 and 5 minutes after exercise. The quantity of lactate after 5 minutes was taken as an indicative measurement. Above 15.3 mmol/l indicates an increased content of lactate, anaerobic endurance.

The volume and fullness of any function is determined by the possibility of energy exchange. Scientific research in the field of clinical and sports physiology has established that one of the parameters that most accurately reflects the state of energy processes in the body is the metabolite of glycolysis-lactate. When performing the limiting short-time strain the high athlete performance is characterized by a high level of concentration of lactic acid. Peak lactate values after 1, 3 and 5 minutes of recovery ranged from 15.3 to 21.3 mmol/l. These data indicate the high anaerobic performance of athletes. It should also be noted that the maximum lactate levels were recorded at different times during the recovery period. High concentration of lactic acid at rather low test indicators testifies to the lowered aerobic working capacity of athletes.

On all studied physiological parameters athletes were divided into groups control and a case (table 1). The distribution of genotypes by polymorphisms studied in the formed groups is presented in table 1.

Table 1 – Distribution of genotypes by polymorphisms 287 I/D *ACE*, R577X ACTN in groups of athletes

№	Parameters	Genotypes (number of people)	***ACE***			***ACTN***		
			I/I	I/D	D/D	R/R	R/X	X/X
1	BMI	Control (BMI < 20 at the 19-24 ages, BMI =20-25 at the 25-34 ages)	0	8	4	3	6	3
		Case	0	6	2	2	3	3
2	LI	Control – (normal limits 75 – 80 ml/kg.)	0	5	0	1	3	1
		Case	0	9	6	4	6	5
3	CSE	Control (athletes with model indexes of CSE 63% and >)	0	5	3	2	6	0
		Case	0	9	3	3	3	6
4	LC	Control (LC=5100 and >)	0	10	6	3	8	5
		Case (LC to 5100 ml)	0	5	0	2	1	2
5	Lactate	Control (<15.3 mmol/l)	0	4	1	0	3	2
		Case (>15.3 mmol/l)	0	9	3	5	4	3
6	Muscle bulk	Control (36 and >)	0	3	3	1	4	1
		Case (< 36)	0	11	3	4	5	5

Table 2 – Polymorphism associations of 287I/D *ACE*, R577X *ACTN* with physiological parameters

Parameters		*ACE* gene I/D polymorphism					
		Control	Case	χ2	*p*	OR	CI
BMI	I allele	0,333	0,375	0,073	0,787	1,20	0,32-4,50
	D allele	0,667	0,625			0,83	0,22-3,12
LI	I allele	0,50	0,30	1,319	0,251	0,43	0,10-1,85
	D allele	0,50	0,70			**2,33**	0,54-10,10
CSE	I allele	0,312	0,375	0,165	0,685	**1,32**	0,35-5,05
	D allele	0,688	0,625			0,76	0,20-2,90
LC	I allele	0,312	0,50	1,167	0,280	**2,20**	0,52-9,36
	D allele	0,688	0,50			0,45	0,11-1,93
Lactate	I allele	0,40	0,375	0,019	0,891	0,90	0,20-4,08
	D allele	0,60	0,625			1,11	0,25-5,04
MB	I allele	0,250	0,393	0,754	0,385	**1,94**	0,43-8,79
	D allele	0,750	0,607			0,52	0,11-2,33
		ACTN gene R/X polymorphism					
		Control	Case	χ2	*p*	OR	CI
BMI	R allele	0,50	0,438	0,150	0,698	0,78	0,22-2,77
	X allele	0,50	0,563			**1,29**	0,36-4,58
LI	R allele	0,50	0,467	0,033	0,855	0,88	0,21-3,66
	X allele	0,50	0,533			1,14	0,27-4,79
CSE	R allele	0,625	0,375	2,406	0,121	0,36	0,10-1,33
	X allele	0,375	0,625			**2,78**	0,75-10,26
LC	R allele	0,438	0,50	0,120	0,729	**1,29**	0,31-5,33
	X allele	0,563	0,50			0,78	0,19-3,23
Lactate	R allele	0,30	0,583	2,267	0,132	**3,27**	0,67-15,82
	X allele	0,70	0,417			0,31	0,06-1,48
MB	R allele	0,50	0,464	0,043	0,836	0,87	0,22-3,36
	X allele	0,50	0,536			1,15	0,30-4,47

The results of statistical analysis of the Association of polymorphisms of *ACE* 287I/D, R577X *ACTN* with physiological parameters using the Yets'correction are presented in table 2. Outlines the following trends: the D allele is associated with lower performance of LI, the I allele with less indicators of CSE, LC, MB.

In respect of *ACTN* gene polymorphism, the R allele correlated with high levels of lactate and less with LC, the X allele with increased BMI, with the worst performance of CSE. However, it should be noted that due to the small sample data are not statistically significant, it is necessary to further study on a larger number of athletes to obtain more reliable data.

Thus, in this paper, a cohort for the study was formed, consisting of 23 players of professional level and an electronic database was created for the study group according to the survey data. Polymorphic loci of *ACE* I/D and *ACTN* R/X genes are genotyped in this cohort.

Due to the small number of control and experimental samples, the obtained data are not statistically significant, but it can be concluded that heterozygous variants of genes are the most common in the study group of football players, which indicates the joint activity of favorable alleles. Athletes in the presence of heterozygous genotype for both genes have both good aerobic and anaerobic performance, due to the

presence of slowly contracting and rapidly contracting muscle fibers, characterized by an optimal combination of speed-strength and endurance.

Thus, our data confirmed previously identified genetic conditionality of many physiological parameters, including most of the metabolic characteristics of the body, aerobic and anaerobic capabilities, the percentage of fast and slow fibers in the muscles and others. This proves the necessity of multi-stage selection of young athletes on the basis of complex testing and analysis of their genetic and physiological parameters.

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СПОРТТЫҚ ІРІКТЕУГЕ АРНАЛҒАН ГЕНЕТИКАЛЫҚ ӘДІСТЕРДІ ҚОЛДАНУ АРҚЫЛЫ ҚАЗАҚСТАН ФУТБОЛШЫЛАРЫНЫҢ ФИЗИОЛОГИЯЛЫҚ КӨРСЕТКІШТЕРІ МЕН I/D *ACE* ЖӘНЕ R/X *ACTN* ГЕНДЕРІ ПОЛИМОРФИЗМДЕРІ АРАСЫНДАҒЫ БАЙЛАНЫСТАРДЫ ЗЕРТТЕУ

Аннотация. Жұмыста молекулалы-генетикалық талдау және негізгі физиологиялық көрсеткіштер деңгейлерін зерттеу негізінде I/D *ACE* және R/X *ACTN* гендері полиморфизмдерінің кәсіби Қазақстандық футболшылардың дене шынықтыру дайындығы арасындағы байланыстарына талдаулар жүргізілген. Алынған нәтижелер аталған гендердің полиморфизмдері спорттық іріктеуде тиімді қолдануға болатындығын дәлелдейді.

Түйін сөздер: спорттық іріктеу, молекулалы-генетикалық маркерлер, ген полиморфизмі, физиологиялық көрсеткіштер, антропометрия, волюмоспирометрия, биоимпедансометрия (Танита), хронометрия, лактометрия.

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ИЗУЧЕНИЕ АССОЦИАЦИИ ПОЛИМОРФИЗМОВ I/D *ACE* И R/X *ACTN* С ФИЗИОЛОГИЧЕСКИМИ ПАРАМЕТРАМИ ФУТБОЛИСТОВ КАЗАХСТАНА ДЛЯ РАЗРАБОТКИ ГЕНЕТИЧЕСКИХ МЕТОДОВ СПОРТИВНОГО ОТБОРА

Аннотация. В научно-исследовательской работе на основе молекулярно-генетического анализа и изучения основных физиологических параметров был проведен корреляционный анализ между генотипами полиморфизмов I/D *ACE* и R/X *ACTN* и уровнем физической подготовленности казахстанских футболистов профессионального уровня. Полученные результаты свидетельствуют о перспективности применения данных полиморфизмов для разработки генетических методов спортивного отбора.

Ключевые слова: спортивный отбор, молекулярно-генетические маркеры, полиморфизмы генов, физиологические параметры, антропометрия, волюмоспирометрия, биоимпедансометрия (Танита), хронометрия, лактометрия.

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THE PROBLEM OF UNUTILIZED AND BANNED PESTICIDES IN KAZAKHSTAN (review)

Abstract. The article presents a review about the problems of obsolete and dangerous pesticides in the world and in Kazakhstan. The huge production of pesticides, including persistent organic pollutants (POPs), it's excessive supply by agro-industrial companies, the socio-economic restructuring of entire agricultural infrastructure in CIS countries associated with the liquidation of collective farms, and processes of land privatization led to the accumulation of tons of obsolete pesticides in the global scale. Unutilized pesticides are stored in dilapidated or destroyed warehouses, and they can get into the environment with rain, wind, floods, landslides and fires. The contradictory results of obsolete pesticides inventory in Kazakhstan indicate that a scientific approach is necessary for a careful analysis of contamination foci and the development of methods for reclamation of territories contaminated by pesticides. According to the World Health Organization (WHO), active ingredients of some pesticides represent a risk of acute influence on health. Among the 57 pesticides which were approved for use in the country, 29 pesticides are hazardous. In this regard, it is necessary to take measures to stop the import of severely hazardous pesticides and do the utilization of banned POPs.

Key words: obsolete pesticides, unutilized pesticides, reclamation, environment.

Introduction. The industrialization of the agriculture has increased the chemical load on natural ecosystems. Intensive chemicalization of agriculture has led to an increase in yields, but, at the same time, to the pollution of the environment by pesticides and other chemical compounds. The use of pesticides is claimed by the commercial interest of industrial agricultural production. Pesticides are used to control insect pests (insecticides) and various parasites, plant diseases, pathogenic fungi (fungicides), weeds (herbicides), warm-blooded pests of seeds and grain products (zoocides), wood, as well with transmitters of dangerous human and animal diseases. For today it is necessary to ascertain the economic feasibility of using in agriculture the chemical plant protection products and to forecast further increase of their imports, expansion of the sphere of application and rapid renewal of the assortment. For example, in Cameroon, tomato yield losses from pest and diseases are high and can reach 100%, if the crop was not treated by insecticides [1].

In the world, the use of pesticides and the volume of pesticide production are increasing from year to year. Approximately 2.3 million tons of industrial pesticides are used annually. This is due to the need of maintenance the food for the growing world population [2]. For example, 13.1 kg/ha of pesticides are

used in Japan, 59.4 kg/ha - in the Bahamas, 8.8 kg/ha – in the Netherlands. In Argentina, Bangladesh, Thailand, Brazil, Chile, China and Canada, more than 20 million kilograms of pesticides were used annually between 1990 and 2012 [3].

Although areas of agricultural land were decreased between 2011 and 2015, the volume of chemical plant protection products application was not reduced Currently, the area of agricultural land in Kazakhstan is about 21 million hectares. Specific application of pesticides per 1 hectare of agricultural land has increased almost 2 times (from 0.29 kg/ha in 2011 to 0.52kg/ha in 2015). During this period, the annual volume of introduced pesticides varied between 10 and 11 thousand tons. A total of 51,154.7 tons of pesticides were introduced in the last five years [4].

Hazardous pesticides. According to the World Health Organization (WHO), "hazardous pesticide" is defined as a pesticide that represents the risk of acute influence of Human health. In recent years, the term " highly hazardous pesticides" (HHP) has been expanded to include not only pesticides with acute toxic effects, but also those pesticides that cause serious chronic effects on human health. Scientific data on chronic health effects of pesticides are constantly being updated by WHO/UNEP. The active substances of pesticides affect the endocrine system and have a carcinogenic effect. Among them - pesticides from the lists of the Stockholm Convention on POPs and the Rotterdam Convention.

Due to official data, only drugs permitted for use are imported into Kazakhstan. But nevertheless, it turned out that out of 57 pesticides the active substances of 29 pesticides are classified as hazardous according to the PAN list (Pesticide Action Network list dated by January 16, 2009; table 1).

These dangerous preparations were widely used in 2003-2012 to control pests, diseases and insects. During the comparison of the list of pesticides allowed in EECCA (Eastern Europe, Caucasus and Central Asia) countries with the list of OOP (Object-oriented programming) prepared by IPEN, from 32 names in Ukraine to 10 in Belarus were found up. Conducted studies present only approximate information on the OOP. Therefore, it is necessary to take measures to stop the import and use the highly hazardous pesticides. What is their quantity on the territory of our country? And what is their real danger? This issue remains open.

One of the most dangerous pesticides are persistent organic pesticides (POPs). POPs are chemicals that contain chlorine, carbon and hydrogen. POPs are the group of toxic chemicals that gather in the environment, accumulate in the fatty tissues of living organisms and humans, causing irreparable health disturbances. POPs are not destroyed in the environment for a long time, transported by air and water masses over long distances, far from the original pollution site [5, 6].

In 2001, the Stockholm Convention was developed by world community concerned with the crucial state of environmental pollution by persistent organic pollutants. The main aim of the Stockholm Convention, which was signed by 151 countries. is to limit or stop the production and use of all intentionally produced POPs, i.e. chemicals and pesticides. The Convention also provides the progressive minimization and, as far as possible, the final cessation of unintentionally produced POPs releases, such as dioxins and furans. Implementation of the Convention will lead to the fact that the production and use of POPs will be discontinued, their stocks will be disposed, and, what is especially important, the introduction of new POPs into the environment will be prevented. Kazakhstan joint to this Convention, therefore, undertaking the obligations not to produce, not to use, or destroy stocks of chemicals deemed particularly hazardous to life.

12 chemical substances, of which 9 are pesticides (DDT, aldrin, dieldrin, endrin, chlorodan, heptachlor, mirex and toxaphene) came under the jurisdiction of the Convention. The list of POPs is constantly enriched with new substances. For example, at the fourth meeting of Parties of the Conference, which was held in 2009, nine additional chemicals were included to POPs list, including five pesticides (chlordecane, α-hexachlorocyclohexane, β-hexachlorocyclohexane, lindane, pentachlorobenzene). To the state of 2013, the list of POPs included 14 names of organochlorine pesticides [7]. In 2015, the list included brominated flame retardants and associated with them precursors as perfluorinated alkylated substances [8].

The persistent organic pollutants (POPs), are particularly dangerous among call chemical pollutants of the environment [9, 10]. The POPs problem has recently become particularly acute among a number of global environmental threats. POPs compounds are difficult to decompose and accumulate in living organisms. POPs are transported over long distances through air, water, and the insects, birds and migrating

Table 1 – Pesticides, which were included in the list of highly hazardous pesticides (2009)

Name	Classification	Active substance	Toxic effect
Pesticides from the class of organophosphorus pesticides			
Dursban	Insecticide	Chlorpyrifos	Very toxic
Sumithione	Insecticide	Fenitrothion	Carcinogen category 1
Quikfos	Fumigant	Phosphine	Very toxic
Nurell-D	Insecticide	Cypermethrin	Carcinogen, toxic
Phosphamide	Insecticide	Dimethoate	Carcinogen category 1
Pesticides from the class of organochlorine pesticides			
Daconyl	Fungicide	Chlorothalonil	Carcinogen, toxic
Sportak	Fungicide	Prochloraz	Bioaccumulative, toxic
Diphezan	Herbicide	Chlorsulfuron	Bioaccumulative, toxic
Dual	Herbicide	Metalochlor	Bioaccumulative, toxic
Cross	Herbicide	Chlorsulfuron	Bioaccumulative, toxic
Oktigen	Herbicide	Chlorsulfuron	Bioaccumulative, toxic
Trophy	Herbicide	Acetochlor	Carcinogen category 1
Trophy-super	Herbicide	Acetochlor	Carcinogen category 1
Pesticides from the class of synthetic peritroids			
Buldock	Insecticide	Beta-siflutrin	Very toxic
Fastak	Insecticide	Beta-siflutrin	Very toxic
Kinmiks	Insecticide	Beta-siflutrin	Very toxic
Politrin	Insecticide	Cypermethrin	Possible carcinogen
Sherpa	Insecticide	Cypermethrin	Possible carcinogen
Karate	Insecticide	Lambda-cyhalothrin	Endocrine system destroyer
Ustad	Insecticide	Cypermethrin	Possible carcinogen
Cyrax	Insecticide	Cypermethrin	Possible carcinogen
Cytcore	Insecticide	Cypermethrin	Possible carcinogen
Rovikurt	Insecticide	Permethrin	Carcinogen category 1
Atilcord	Insecticide	Cypermethrin	Possible carcinogen
Decil	Insecticide	Deltametrin	Endocrine system destroyer
Pesticides from the class of metal-containing compounds			
Methyl bromide	Insecticide	Methyl bromide	Carcinogen category 1
Pesticides from the class of simm-triazine pesticides			
Gezagard-50	Herbicide	Prometrin	Carcinogen category 1
Lentagran -Comby	Herbicide	15% atrazine	Carcinogen category 1
Pesticides from a new class of imidazoline pesticides			
Pivot	Herbicide	Imazetapir	Toxic

warm-blooded animals contribute to it. Even in small doses, POPs pose a real threat to human and nature. POPs are slightly soluble in water and are highly soluble in fats (oils), that's why POPs accumulate in the fat tissue of living organisms. Their POPs concentration can increase in thousands and tens of thousands of times moving along the food chain.

Persistent organic pesticides are characterized by the following features:

– they remain in the environment for a long time until their full decomposition;

– they transmitted over long distances, even to areas far from thousands of kilometers from their emission sources;

– they are accumulated in the tissues of all living organisms penetrating with food, drinking water or atmospheric air;

– they poison human and animals, causing a wide spectrum of toxic disturbances;

– they have a mutagenic, teratogenic or carcinogenic effect;

– they can also concentrate as they move along the trophic chain: "soil - plant - animal - human" locally increasing the accumulation level in the body.

The ratification of the Stockholm Convention by the Republic of Kazakhstan shows that the country turned to integration into the worldwide process of cooperation in the field of human health care and improve the environment quality (The Law of the RK was ratified on June 7, 2007, № 259-III). Kazakhstan took obligations not to produce, not use, and also dispose the stocks of chemicals that are considered especially dangerous for human life. Kazakhstan is a member of international cooperation and legislative acts of the Republic of Kazakhstan in the field of environmental protection:

– Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal. Ratified by the Law of the RK on February 10, 2003. № 389-II;

– Rotterdam Convention on the procedure of preliminary informed agreement on separate hazardous chemicals and pesticides in international trade. Ratified by the Law of the RK on March 20, 2007;

– Instruction "On the procedure for disposal or destruction of proscribed and degraded pesticides and packaging", 1996;

– the laws "On Environmental Protection", "On Subsoil and Subsoil Use, 1997;

– President of the Republic of Kazakhstan issued a decree "On the concept of ecological security of the Republic of Kazakhstan for 2004-2015, - 2003;

– Ecological Code of the Republic of Kazakhstan, Law of the Republic of Kazakhstan № 212-III. 2007.

Obsolete pesticides. Pesticides become obsolete and undesirable when they are not suitable for use because of: unsatisfactory storage conditions that lead to damage or change in chemical composition or loss of product properties; expiration of the shelf life; prohibition of use and other changes relating to product registration and permit for its use [11].

The huge production of pesticides, including POPs, it's excessive supply by agro-industrial companies, the socio-economic restructuring of entire agricultural infrastructure in CIS countries associated with the liquidation of collective farms, and processes of land privatization led to the accumulation of tons of obsolete pesticides in the global scale [12, 13]. According to the International HCH & Pesticides Association, the exact amount of obsolete pesticides in the countries of the former Soviet Union has not been established and data vary greatly [8]. In 2007, the quantity of obsolete pesticides in Belarus amounted to 6558 tons, and 20,000 tons were inventoried in frame of the project GCP/RER/ 040/EU. In 2005, the registered quantity of obsolete pesticides for Uzbekistan was 17,718 tons, but later the UNEP/POPs/INC.5/1 project reported about 40,000 tons. It is known, that in the Russian Federation stocks of obsolete pesticides, which left over from the Soviet times, usually located near the former collective farms, and often in the vicinity of remote villages or forestry [14]. In Ukraine, a preliminary inventory has identified more than 3,000 pesticide stores, including nearly 2,000 destroyed warehouses, in which stored about 33,000 tons of obsolete pesticides [15].

In Kazakhstan, pesticides with properties of POPs have never been produced, they are not currently imported or exported. Export and import of POPs containing pesticides are prohibited in accordance with the legislation of the Republic of Kazakhstan. But, significant amounts of previously produced POPs, which were used in the former USSR, have been stored in Kazakhstan. The main sources of pollution by POPs in Kazakhstan there are:

1) obsolete and unusable pesticides (including those with POPs properties) in agriculture;

2) equipment containing PCBs (polychlorinated biphenyls) used in industry and transport;

3) use in industry of technologies leading to unintentional release of dioxins and furans;

4) the formation of dioxins and furans in the process of open burning.

Foci of soil pollution by residues of POPs containing pesticides are numerous and chaotically distributed throughout the country. Only 20% of the country's territory covers the sites of POPs containing pesticides inventory [4]. Due to the lack of a full-scale inventory in the Republic of Kazakhstan, data on the number of storage facilities, as well as on the quantity and quality of obsolete pesticides, are contra-

dictory. For example, according to one data there are 974 warehouses, 411 of which are in an emergency condition [16]. According to other information [17], there are 1280 warehouses, out of them in emergency condition - 236. The Department of environmental protection in Almaty region considers that 87 tons of obsolete pesticides are subject to utilization, whereas, according to the Ministry of Agriculture, only 126 tons should be destroyed [17]. According to UNEP (data of 2004), in result of inventory of obsolete pesticides, in Kazakhstan, more than 1,500 tons of banned, unusable pesticides and their mixtures of unknown composition were registered. 2008 data indicate that their number reached 10,000 tons [18, 19].

According to the Ministry of Agriculture RK data, reported in July 2012, about 6,931.4 tons of obsolete, banned and unsuitable for use of pesticides are still stored in warehouses of various regions of the country [19].

During the inventory, done in 2009-2010 in the frame of the scientific international program, 64 storage facilities of plant protection chemicals were detected in 10 districts of Almaty region. There were accumulated 68443 kg of obsolete and unusable pesticides. Among them, there were 350 kg of prohibited pesticides (saifos, metaphos), 35543 kg of pesticides with etiquettes and 32550 kg of pesticide mixtures of unknown composition, that consists 79% from total number. There were detected the pesticides from the classes of simm-triazine (atrazine, protrazine, propazin, ziazin), organophosphorus (saiphos, metaphos), chlorine-containing (nitrophene and illoxane), fluorine-containing (treflan), thio-carbamate (temik), as well as pesticides of German and Chinese origin (50% Thirams and Hataonyag).

POPs containing pesticides were not detected during this inventory. However, the results of organoclorine pesticides determining in soil samples, which were taken around 64 former storage facilities of pesticides, have demonstrated that soil around 24 former repositories was contaminated by metabolites of 2,4 DDD; 4,4 DDD; 4,4 DDT; 4,4 DDE and isomers of hexachlorane (α-HCH, β-HCH and γ-HCH. The most polluted regions were Eskildinsky, Talgar, Karasai, Enbekshi-Kazakh districts. The main pollutants were lindane, β-isomer of hexachlorane and metabolites 4,4'DDE and 4,4'DDT. In addition to these metabolites, in soil samples from many regions, there were α-HCH, 4,4-DDD and 2,4-DDD. According to normative documents of Kazakhstan, their presence in the soil is unacceptable [20, 21]. It is known that they are highly toxic agents with expressed skin-resorption toxicity. They cause mutagenic, antimitotic and embryotoxic effects [22].

Despite an inventory based on official figures by the Ministry of Environment of the Republic of Kazakhstan, Ministry of Agriculture of the Republic of Kazakhstan, non-governmental organizations and the Republican Sanitary-epidemiological Station Agency for Health Affairs of the Republic of Kazakhstan, unfortunately, at present there is no complete information on the use of pesticides in our country.

The causes:

– no complete information on the use of pesticides in our country; no waste management strategy in the state scale;

– absent irrational agriculture management methods, which have become the norm for farms and enterprises,

– insufficient regulatory framework;

– lack of economic incentives for the liquidation of waste disposal sites

– no the objective scientific information on the extent of soil contamination with obsolete pesticides, the locations of the former warehouses of chemical plant protection products in different geographical zones of Kazakhstan;

– no the modern laboratory capable of identifying a mixture of obsolete pesticides at the level of international standard;

– no the reliable data on what classes of obsolete pesticides and in what quantities were not buried and disposed of in various region of Kazakhstan.

Impact of obsolete pesticides on the environment. The vast majority of pesticides are poisonous chemicals that poison the target organisms. First of all, the potential danger of pesticides for the environment is explained by the fact that the overwhelming majority of synthetic chemical substances are unnatural. Among all pollutants of nature, the synthetic chemicals are ranked in top ten. They are consciously introduced into the biosphere by a human. And, the scale of introducing of synthetic compounds to the nature increases.

Growth inhibitors and sterilizers, substances causing infertility are also used as pesticides. Under the pesticides influence, some of the biological reactions are stopped, and this allows to human fight the plant diseases, keep food longer, and destroy pests [9]. But a significant decrease of such products qualities as trace element compositions, usefulness and consumer health safety are not taken into account. As a result, the destruction of biocenoses in areas where pesticides are used become the global problem [9, 10].

Unlike other pollutants (radionuclides, heavy metals, etc.), the real danger of pesticides is not fully understood. This is because pesticides are the hundreds of active substances and tens of thousands drugs. The methods of their analysis in the environment are complex, expensive, laborious, imperfect and not always reliable. The lack of information on pesticides ecotoxicological properties is the main reason for their danger. The long-term environmental consequences of pesticides usage have not been studied yet. Analysis of research results regarding the pesticides genetic activity in model test systems has shown that many of them are mutagens [23-25]. Of the 400 studied pesticides, 262 substances (65%) showed the mutagenic activity at any test objects. We should understand that pesticides mutagenicity has a tendency to increase with more number of test systems used because of pesticides synergistic effect [25].

The main problems associated with the use of pesticides in agriculture are their resistance in soil and toxicity to non-target organisms [26] that expresses even at low concentrations [27].

The large-scale use of chemicals to support agriculture adversely affects human health and the environment, depletes the natural resource agriculture base because 60-95% of introduced pesticides do not directly suppress objects, thereby damaging the environment [28, 29].

The doses of a number of pesticides used in agriculture are similar to chemical mutagens, which cause a negative genetic effect. Thus, when studying 407 pesticides for mutagenicity, 263 of them had mutagenic activity and represented a genetic hazard [30].

Posing specific biological activity, pesticides cause not only the death of those organisms against which they were applied, but also cause disruption of vital processes of other organisms, including humans, animals and plants [31, 32]. Genetic examination of persons who have professional contact with pesticides has shown that many pesticides (ciram, zineb, TMTD, benomyl, polychloroprene, polychlorochamber, katoran, etc.) significantly increase the frequency of chromosomal aberrations in peripheral blood lymphocytes in people contacting with pesticides [24, 33].

Pesticides are widely used in agriculture because they can save up to 40% of the crop from losses. However, due to violations of storage technologies and usage, up to 95% of introduced pesticides do not reach the suppressed targets and cause the huge damage to the environment. Application of pesticides without taking into account the natural and climatic conditions of the territories under treatment, the violation of the pesticides management protocols, including rejection of necessary safety measures, creates the serious problems, such as - reducing biodiversity; the death of wild animals and livestock, poisoning; violation of natural control over the different pests quantities; accumulation of a significant amount of obsolete unusable chemicals which are the dangerous sources of environmental pollution; the ingestion of pesticides residual amounts in feed and food; contamination of surface and ground waters. The contaminated food, feed and drinking water are the main sources of pesticide intake into the human body.

The main problem of contaminated soils is the danger to human health from accidental contamination of polluted soil. This is acute question, especially for children. Concern for human health and the environment is mainly related to the long-term effects of certain substances. Endocrine disorders, violations in reproductive function and carcinogenicity are the most often noticeable long-term effects [27].

Long-term effects of pesticides, especially at low doses, and their possible synergism with other environmental pollutants and disease transmitters have been poorly studied due to the relative novelty of most pesticides. However, metabolites of pesticides that remain in food cannot cause the toxic or lethal effects, but they reduce the resistance to diseases and gradually accumulate in the body to a dangerous level [34].

Mutagenic activity of pesticides is one of the most dangerous manifestations of negative influence on human health and its offspring. In the past 10 years, research of pesticides effects on human health has been actively conducted [32-37]. It was proved that some pesticides are carcinogens and cause the development of various cancer types [32, 38]. Pesticides often cause allergies, diathesis and respiratory diseases [39, 40]. Many pesticides are associated with the neurodegenerative diseases development

[32, 35, 41, 42], such as Alzheimer's and Parkinson's. Endocrine diseases [34], problems of female and male reproductive systems [43, 44], type 2 diabetes mellitus [45], metabolic syndromes and obesity, developmental disorders [45] are not a complete list of pesticides impacts on human health. The congenital malformations, which constitute an essential part of the overall morbidity and mortality, are the most serious deviations in children health status. The intrauterine effect of some pesticides increases the risk of lung diseases development in future of child, while the risk of the disease in children increases in direct proportion to the concentration of the pesticide in the mother's blood during pregnancy. Harm from pesticides to children's body is manifested in hyperactivity in children (ADHD), autism.

Multiple effects of pesticides on public health were the reason of fact, that in 2017, UN experts announced the falsity of the claim that pesticides should be used to ensure food safety. Data on 200,000 fatal poisonings by pesticides per year and evidence of permanent contact with pesticides is associated with a wide range of diseases, developmental disorders and sterility were presented [46].

Polish scientists A. Buczynska and I. Szadkowska-Stanczk [47] studied the impact of pesticide release on the population living near these places and assessed their impact on health. Out of the 286 pesticide discharge sites in Poland, 40 were selected as the largest source of environmental hazard. In one of the investigated sites, the level of exposure of 2,4-D to the population caused nephrotic and hepatotoxic effects, as well as reproductive disorders. Studies have shown that, even four years after the discharge site was closed, the content of pesticide residues in groundwater was still high.

In today's world, threats to ecosystems and biodiversity are multidimensional: from localized loss of habitats from pollution to the global consequences of climate change. Existing environmental risks from obsolete pesticides stocks are soil degradation, migration of pesticides from contaminated soil to groundwater; contamination of surface waters, migration of pesticides to air by volatilization; the spread of pesticide dust or pesticide-contaminated soil particles by wind and large-scale spreading as a result of natural and emergency disasters such as hurricanes, earth-shaking and flooding.

Recultivation of territories contaminated by obsolete pesticides. Cleaning the soil from obsolete pesticides is a complex and expensive process. As usual, obsolete pesticides are burned in high-temperature furnaces, or buried in special burial grounds. These large-scale and costly rehabilitation technologies are all-effective. But in developing countries, they are not available due to limited financial resources. Despite this, in the frame of ASP program (2003), a large quantities of pesticides were completely or partially destroyed in a number of African countries (Egypt, Namibia, Niger, Senegal, Seychelles, South Africa, Sudan, Tanzania, Uganda, Zambia) in Eastern Europe and in some countries of the former USSR [48].

Moldova was one of the first countries of the former Soviet Union which considered its legacy of obsolete pesticides [49]. Since 2005, this country has been active in the utilization of obsolete pesticides with the support of the Global Environment Facility/World Bank (2006-2010). About 3,350 tons of obsolete pesticides were repackaged, of which 1,292 tons were burned in high-temperature ovens abroad. In 2011-2012, an additional 200 tons of obsolete pesticides were burned in the Czech Republic.

In Armenia, 7100 tons of POP wastes in the form of highly polluted soil, 1050 tons of POPs containing pesticides and 12.7 thousand tons of other obsolete pesticides were burned in high-temperature furnaces. Tons of soils which less polluted by POPs were buried with the support of FAO. Within the framework of the Arctic Council's Action Plan for the Elimination of Arctic Pollution, about 2000 tons of POPs pesticides were repackaged in North-West Russia [14]. In 2010-2013, 10000 tons of hexachlorobenzene from the west of Ukraine was transported to Poland and Germany for burning [50]. Along with physical methods, expensive chemical tools are used, such as immobilization or soil washing. But they are not suitable for large-scale soil restoration activities [51-53].

However, for many CIS countries, including Kazakhstan, the issues of the obsolete pesticides utilization remain open. Taking into account the dangerous of obsolete pesticides and the need of their utilization or disposal, certain legislative acts have been accepted in Kazakhstan [53]. Despite these measures, the problem of pesticides utilization remains topical.

There are main reasons:

– lack of management and objective scientific evidence on the degree of soil contamination by obsolete and unusable pesticides in various geographic areas of Kazakhstan;

– there is no reliable data on the location of the destroyed warehouses, where previously, before 1991 (before the collapse of the USSR), pesticides including POPs were stored.

The agrarian sector is dominant in Kazakhstan. The regions supply agricultural products (vegetables, fruits, meat, milk, etc.) not only in large megacities and its neighborhood, but all industrial and rural districts of Kazakhstan. Obsolete pesticides sites are dangerous sources of environmental pollution. And the urgent measures should be taken to characterize them and then eliminate them.

In this connection, since 2018, within the framework of the MES KN RK scientific-technical program "Comprehensive assessment of unutilized and banned pesticides impact on genetic status and health of population of Almaty region" (BR05236379), a cadaster of obsolete stocks and locations of former storage pesticides is being developed. The program implementation plan based on usage a multi-level approach including: detection of POPs decay products in soil, water, agricultural products; testing it for mutagenicity/genotoxicity in model experiments; reveal the genetic effects on living systems of different organization levels; test the population health status; conduct the molecular genetics and cytogenetic analysis of population, estimate the genetic risk for human health and future generations, and develop the practical and preventive recommendations. Thus, the complex evaluation of long-term influences of obsolete pesticides to the biocenoses and components of the human food chain will be obtained taking into account the health risk to population living in the Talgar district Almaty region.

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ҚАЗАҚСТАНДА ЕСКІРГЕН ПЕСТИЦИДТЕР МӘСЕЛЕСІ (шолу)

Аннотация. Мақалада әлемдегі және Қазақстандағы ескірген және қауіпті пестицидтердің мәселелеріне әдебиеттік шолулар жасалған. Пестицидтердің, оның ішінде тұрақты органикалық ластағыштардың (ТОЛ) шамадан тыс көптеп өндірілуі, агроөнеркәсіптік компаниялардың шектен тыс қолдануы, ТМД елдерінде, оның ішінде Қазақстанда ауыл шаруашылық инфрақұрылымды құрайтын колхоздар мен совхоздардың жабылуы және Жерді жекешелендіру жұмыстары ескі қолданылмаған пестицидтердің үлкен көлемде жинақталуын тудырды. Бұл жарамсыз пестицидтер қазіргі кезде бұзылған қоймаларда сақталуда және олар сыртқы ортаға жауын сулары, жел, су тасқыны немесе басқалары арқылы таралуы мүмкін. Ескі пестицидтерді инвентаризациялау бойынша қарама-қарсы нәтижелердің болуына байланысты ластанған аймақтарды талдау және топырақ құнарлығын қайта қалпына келтіруге ғылыми тұрғыдан амалдар қажет. Ескі пестицидтермен қатар қазіргі кезде қолданылатын пестицидтердің өзін Дүниежүзілік Денсаулық Сақтау Ұйымы (ДДСҰ) адам денсаулығына қауіп келтіретінін көрсетіп отыр. Қазақстан Республикасында қолдануға рұқсат етілген 57 пестицид түрінің 29-ы қауіпті деп саналады. Осыған байланысты, қауіпті деп танылған пестицидтерді шеттен әкелуге тыйым салу керек.

Түйін сөздер: ескі пестицидтер, қауіпті пестицидтер, топырақ құнарлығын қайта қалпына келтіру, қоршаған орта.

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ПРОБЛЕМА УСТАРЕВШИХ ПЕСТИЦИДОВ В КАЗАХСТАНЕ (обзор)

Аннотация. В статье представлен литературный обзор о проблемах устаревших и опасных пестицидов в мире, в том числе Казахстане. Огромное производство пестицидов, в том числе стойких органических загрязнителей (СОЗ), чрезмерная закупка агропромышленными компаниями социально-экономическая перестройка, связанные с ликвидацией колхозов и совхозов, приватизацией земель, а также всей инфраструктуры

сельского хозяйства странах СНГ, в том числе Казахстане привело к накоплению тонны устаревших пестицидов в глобальном масштабе. Устаревшие пестициды хранятся в ветхих либо разрушенных складах, и они могут попадать в окружающую среду с дождем, ветром, в результате наводнений, оползней и пожаров. Противоречивые результаты инвентаризации устаревших пестицидов в стране свидетельствуют о том, что необходим научный подход для тщательного анализа очагов загрязнения и разработке методов рекультивации территорий, загрязненных устаревшими пестицидами. Наряду с устаревшими пестицидами среди используемых пестицидов согласно Всемирной организации здравоохранения (ВОЗ) действующие вещества некоторых пестицидов представляют риск острого воздействия на здоровье человека. Среди разрешенных к применению 57 наименовании пестицидов в стране действующее вещество 29 препаратов относятся к опасным. Необходимо принять меры по прекращению импорта особо опасных пестицидов.

Ключевые слова: устаревшие пестициды, опасные пестициды, рекультивация, окружающая среда.

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BIODIVERSITY OF DIATOMS ALGAE OF ALAKOL LAKE AND ITS SYSTEMATICS

Abstract. In Kazakhstan, there are many specially protected natural territories: nurseries, national parks, reserves, sanctuaries, wildlife areas, natural monuments, botanical gardens established for the preservation of biological diversity of the state. In many of those areas, the scientists-florists conducted scientific research related to the inventory of vascular plants. Despite the substantial interest for the study of flora, the research into their diversity in various natural communities is insufficient, especially the flora of water reservoirs. The algae of water reservoirs remain studied to a small extent.Nevertheless recently we have conducted the study of algae flora in the specially protected natural territories of various regions of Kazakhstan. In the article, the authors provide research data's for the first timeinvestigate of the algal flora of Alakol lake, which flows through 15 rivers (the Urzhar, the Katynsu, the Emelkuisa, the Yrgaity, the Zhamanty, the Zhamanotkel, the Tastu etc.). The found seaweeds were divided into: 1-systematic division, 3-classes, 13-orders, 23-families, 103-species and species belonging to 41-genures. Biodiversity of specially established types of seaweed has developed and modern taxonomy has been created. In the studied lake of the algae are found cosmopolitan species in different areas [2-5]. Most of the species listed here are of the plankton bacterial species and some species are of benthos.

Key words: algae, plankton, benthos, systematics, lake Alakol.

Introduction. TheLake Alakol is a saline drainage lake located on the Balkhash-Alakol lowland, which is located on the border of the Almaty and East Kazakhstan regions, in the eastern part of the Balkhash-Alakol Basin.More than 15 tributaries flow into the lake, of which the main are the rivers Urzhar, Katynsu, Emelkysa, Ygrajty, Zhamanty, Zhamanotkel, Tasty.The area of the lake (with islands) is 2696 square kilometers. The volume of water is 58.56 cubic km. Length-104 km. Width-52 km. Average depth-22 m. The greatest depth is 54 m.The length of the coastline is 348 km.

Together with the lakes Sasykkol, Uyaly, Zhalanashkol and others, smaller, they form the Alakol lake system.In the center of Alakol, there are islands: Ulken, Kishkeni Araltobe, Belkuduk, etc. The climate of the coast is sharply continental. A complex wind regime is observed above the lake. The maximum wind speed over the northern parts of the lake reaches 40-50 m/s, over the southeastern and central 50-60 m/s. The most active winds in the autumn-winter period, when the wave height can be up to 2-2,5 m.

The duration of freeze-up is about 2 months (February-March). The largest thickness of ice is 0.8 m (in February). Melting ice-April-early May. The water temperature reaches +7+ 15^0C in late May. Mineralization of water in the water varies from 1.2 to 11.6 g/l. The composition of water is chloride-sodium and chloride-sulfate-sodium. In the waters of the Lake Alakol, the high content of fluorine and bromine. In 1994, the Parliament of Kazakhstan ratified the Convention on Biological Diversity, thus affirming its desire to preserve the unique richness of nature. A real step towards the implementation of these documents was the creation in 1998 of the Alakol State Reserve.

Material and methods

The material of this article is selected 2016-2017. During the summer expedition time, a species was collected from different points of the Alakol lake. Along the collection of algae, meteorological conditions of the water, air and water temperature were determined. The water depth is determined by the Sekki disk, water ph- universal indicator paper. The water temperature showed the sample at 22°C, and the water was Ph-7.5.In the course of the work, commonly known classical methods of hydrobotanics and algae were used. To determine phytoplankton samples is a specific examination by M. Gollerbach and B. N. Polyansky, also by the method of N. P. Masiuk and others use Apshtain netting with diameter 45 cm is filtered by plankton grid number 76 [1, 2]. The collected material was fixed there in 4% solution of formalin and 96% etanol. During harvesting, the algae type, color, colony, etc. p. signs are logged. 26 algae samples from plankton, periphyton, and benthos were collected from the lake. Diatomic algae preparations are investigated by heating. Formalin-treated material is coated with glass and heated in the electric cooker. Final preparations are used to identify the types of algae diatoms. Organic cleaning of algae piglets is carried out by firing in strong acids [6-13].

In the identification of species, light microscope MBI-3 and binoculars were produced using a computer program with the binoculars Motic BA 400 microscope, and the size of the cells was obtained by using an ocular micrometer.

Results and discussion

As a result of processing algae samples collected from Lake Alakol, analysis of algae obtained from the lake was investigated and modern systematic groups were identified.They are as follows:

1-division (*Baccillariophyta*), 3-klass (*Baccillariophyceae, Coscinodiscophyceae, Mediophyceae*), 13-order (*Thalassiophysales, Aulacoseirales, Naviculales, Cymbellales, Cocconeidales, Stephanodiscales, Licmophorales, Tabellariales, Surirellales, Fragilariales, Rhopalodiales, Bacillariales, Mastogloiales*), 23- family (*Catenulaceae, Aulacoseiraceae, Cymbellaceae, Naviculaceae, Cocconeidaceae, Stephanodiscaceae, Ulnariaceae, Tabellariaceae, Gomphonemataceae, Entomoneidaceae, Rhopalodiaceae, Fragilariaceae, Amphipleuraceae, Bacillariaceae, Mastogloiaceae, Neidiaceae, Pinnulariaceae, Pleurosigmataceae, Achnanthidiaceae, Achnanthaceae, Surirellaceae, Stauroneidaceae, Rhoicospheniaceae*), 41-genius (*Amphora, Aulacoseira, Brebissonia, Caloneis, Cocconeis, Cyclotella, Cymbella, Cymbopleura, Craticula, Ctenophora, Diatoma, Encyonema, Entomoneis, Epithemia, Fragilaria, Frustulia, Gomphonema, Hannaea, Halamphora, Gyrosigma, Hantzschia, Mastogloia, Navicula, Neidiomorpha, Neidium, Nitzschia, Odontidium, Pinnularia, Pleurosigma, Planothidium, Platessa, Placoneis, Rhoicosphenia, Rhopalodia, Staurosira, Ulnaria, Surirella, Synedra, Tabularia, Tryblionella, Stauroneis*)the species belong to interdisciplinary forms with the following, 103 - species [13-20].

Type of the Alakol lake algae

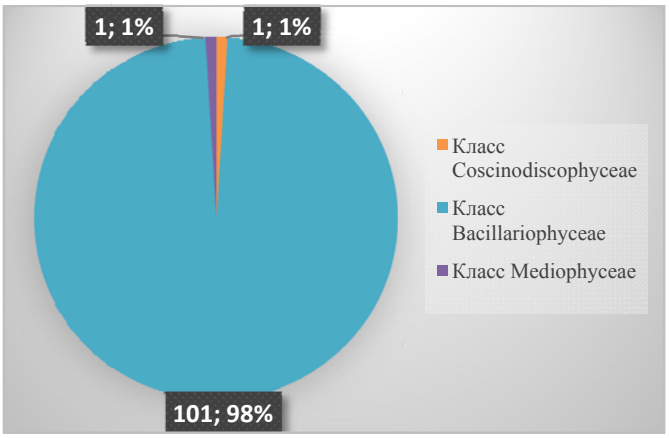
№	Name of species	№	Name of species
1	*Amphora. eximia* J.R.C.	13	*Cym. cistula* (Ehr.) O.Kir.
2	*Am. ovalis* (Kütz) Kütz.	14	*Cym. cymbiformis* C.Ag.
3	*Am. lineolata* Ehr.	15	*Cym. affinis* Kütz.
4	*Am.ambigua* (Grun.) Sim.	16	*Cym. affinis* var. *neoprocera* W.Silva.
5	*Brebissonia lanceolata* (C.Ag.) R.	17	*Cym. helvetica* Kütz.
6	*Caloneis latiuscula* (Kütz.) Cl.	18	*Cymbopleura inaequalis* (Ehr.) Kr.
7	*Cal. westii* (W.Sm.) Hen.	19	*Craticulaambigua* (Ehr.) D.G.Mann in Round.
8	*Cal. amphisbaena* (Bory) Cl.	20	Ctenophora *pulchella* var. *lacerata*(Hust.) Buk.
9	*Cal. amphisbaena* var. *subsalina* (Donk.) Cl.	21	*Cten. pulchella* (Ralfs ex Kützing) D.M.
10	*Cocconeisplacentula* var. *euglypta* (Ehr.) Grun.	22	*Diatomamoniliformis* (Kütz.) D.M.Wil.
11	*Cyclotella meneghiniana* Kütz.	23	*Encyonemaleibleinii (C.Ag.) W.J.Silva.*
12	*Cymbella parva* (W.Sm.) Kirch.	24	*En. silesiacum (Bleisch) D.G.Mann.*

1-table continuation

25	*Entomoneis paludosa var. subsalina (Cl.) Kr. in L.-B.*	53	*Han. arcus* var. *amphioxys* (Rab.) R.M.Pat.
26	*Ent. paludosa* (W.Sm.) R.	54	*Halamphora veneta* (Kütz.) Lev.
27	*Epithemia argus* var. *alpestris*(W.Sm.) Grun.	55	*Hantzschia amphioxys* (Ehr.) Grun.
28	*Epith. argus* var. *angustata* Tarn.	56	*Hant. amphioxys* var. *constricta* Pant.
29	*Epith. adnata* var. *porcellus* (Kütz.) R.Ros.	57	*Mastogloia smithii* Thw. ex W.Sm.
30	*Epith. sorex* Kütz.	58	*Mast. albertii* A.Pav., E.J., C.E.W., L.E. & Z.L.
31	*Epith. turgida* (Ehr.) Kütz.	59	*Mast. pumila* (Grun.) Cl.
32	*Epith. adnata* var. *saxonica* (Kütz.) R.M.P.	60	*Navicula cuspidata* f. *primigena* Dip.
33	*Encyonema silesiacum* (Bl.) D.G.	61	*Nav. pusilla* var. *jacutica* Kis.
34	*Enc. subventricosum* (Chol.) Kr.	62	*Nav. rhynchotella* L.-B.
35	*Fragilaria rumpens* (Kütz.) G.W.F.Car.	63	*Nav. sphaerophora* Ehr.
36	*Frag. capucina* Desm.	64	*Nav. dicephala* Ehr.
37	*Frag. crotonensis* Kit.	65	*Nav. tripunctata* (O.F.Mül.) B.
38	*Frag. acus* (Kütz.) L.-B. in Kr. & L.-B.	66	*Nav. radiosa* Kütz.
39	*Frustulia rhomboides* (Ehr.) De Ton.	67	*Nav. trivialis* L.-B.
40	*Frus. crassinervia* (Br. ex W.Smi.) L.-B. & K.	68	*Neidiomorpha binodis* (Ehr.) M.
41	*Gomphonema constrictum* Ehr. in Kütz.	69	*Neidium productum* (W.Sm.) Cl.
42	*Gom. angustatum* (Kütz.) Rab.	70	*Nei. ampliatum* (Ehr.) Kr.
43	*Gom. olivaceum* (Horn.) Bréb.	71	*Nitzschia acicularis* (Kütz.)W.Sm.
44	*Gom. parvulum* (Kütz.) Kütz.	77	*Nit. vermicularis* (Kütz.) Hant.
45	*Gom. vibrio* Ehr.	78	*Nit. scalpelliformis* Grun.
46	*Gom. calcareum* Cl.	79	*Odontidium mesodon (Kütz.)Kütz.*
47	*Gom. gracile* Ehr.	80	*Pinnularia brauniana* (Grun.) St.
48	*Gom. insigne* W.Greg.	81	*Pin. viridis* (Nitzsch) Ehr.
49	*Gyrosigma acuminatum* (Kütz.) Rab.	82	*Pin. hemiptera* Bréb. ex Gr.
50	*Halamphora veneta* (Kütz.) Lev.	83	*Pleurosigma elongatum* W.Sm.
51	*Hal. coffeiformis (C.Ag.) Lev.*	84	*Planothidiumlanceolatum* (Br. ex Kütz.) Lang.-Ber.
52	*Hannaea arcus (Ehr.) R.M.Pat.*	85	*Platessa salinarum* (Grun.) Lang.-Ber.

1-table continuation

86	*Placoneis elginensis* (W.Greg.) E.J.Cox	95	Synedra *familiaris* Kütz.
87	*Rhoicosphenia abbreviata* (C.Ag.) Lang.-Ber.	96	*Syn. rumpens* var. *scotica* Grun.
88	*Rhopalodia gibba* var. *ventricosa* (Kütz.) H.P. & M.P.	97	*Tabularia fasciculata* (C.Ag.) D.M.
89	*Rh. gibba* (Ehr.) Otto Mül.	98	*Tryblionella levidensis* W.Sm.
90	*Staurosira venter* (Ehr.) Cl.	99	*Tryb. hungarica* (Grun.) Freng.
91	*Stauroneis phoenicenteron* (Nitzsch) Ehr.	100	*Tryb. navicularis* (Bréb.) Ral.
92	Surirella *elegans* Ehr.	101	*Ulnaria amphirhynchus* (Ehr.) Com. & Bukh.
93	*Sur. brebissonii* Kr. & Lan.-Ber.	102	*Ul. amphirhynchus* (Ehr.) Com.
94	*Sur. librile* (Ehr.) Ehr.	103	*Ul. ulna* (Nitzsch) Comp.



Percentage-dimensional indicators of diatomite Alakol lake

Discussing the results, many water reservoirs, alga flora of river lakes in our country have been studied, including the Caspian Sea, Syrdarya, Ili, Baskan and Sarkand, Shar and Kokpekty rivers and algal flora and algal biological diversity of the Alakol lake were not investigated by the country's algal specialists. One of the main objectives of the UN Conference on Biodiversity Conservation, adopted in 1992 in Rio de Janeiro is to preserve biodiversity in the environment and prevent the disappearance of species. The algal diversity of the lake is the basis for this goal. The Kazakh Fisheries Research Institute and the Zoology Research Institute have not studied of Alakol Lake Algapholics byhydrobiotes and ichthyofauna.

During our special algaeological investigations, several times this scientific expedition was built. Algae samples from the northern, southern and south-western parts of the lake were removed and the second part was mixed with 4% solution of formalin and 96% solution of ethanol. A microscopic analysis was carried out to determine the types obtained in the laboratory and the study revealed the varieties of diatomaceous algae and its modern taxonomy. Moreover, we have seen in the study that the Alcohol content of some parts of Lake Alacol Lake is very rich. But in recent years, it can be seen that anthropogenic impact on the stability of lake ecosystems and biodiversity linked to the transformation of the lake into a tourist destination. In this article, the authors regulate the stability of the lake water biota, which is the wealth of algaflora. Consequently, it saves the gaseous, salinity of the water, Ph-levels, mineral composition, and biotic content.

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АЛАКӨЛ КӨЛІНІҢ ДИАТОМДЫ БАЛДЫРЛАРЫНЫҢ АЛУАНТҮРЛІЛІГІ ЖӘНЕ ОНЫҢ СИСТЕМАТИКАСЫ

Аннотация. Қазақстанда көптеген ерекше қорғауға алынған табиғи аймақтар кездеседі: питомниктер, ұлттық саябақтар, қорықтар, жабайы табиғи аймақтар, табиғат ескерткіштері, ботаникалық бақтар мемлекеттің биологиялық әртүрлілігін сақтау үшін құрылған. Осы салалардың көбінде флорист ғалымдар тамырлы өсімдіктерді түгендеуге қатысып, ғылыми зерттеулер жүргізді. Өсімдіктерді зерттеуге үлкен қызығушылық болсада да, әртүрлі табиғатты қорғау қауымдастықтарында олардың алуан түрлілігіне байланысты зерттеулер, әсіресе, су объектілерінің флорасын зерттеу жеткіліксіз. Су балдырларының құрамын зерттеу төменгі деңгейде қалып отыр. Дегенмен альголог ғалымдар Қазақстанның түрлі өңірлерінің ерекше қорғалатын табиғи аумақтарында балдырлар флорасын зерттеуді жүргіздік.Бұл мақалада авторлар 15 өзендер келіп құйатын (Үржар, Қатынсу, Емелқұйса, Ырғайты, Жаманты, Жаманөткель, Тастыт.б) Алакөл көлінің альгофлорасына алғаш рет мәліметтер беріліп отыр. Табылған балдырлар 1 бөлімге, 3 класқа, 13 қатарға, 23 тұқымдасқа, 41 туысқа жататын 103 түрлері мен түр аралық формалары анықталды. Анықталған балдырлар түрлерінің биологиялық сипаттамасы жасалып, заманауи систематикасы жасалынды. Зерттелуші көлден анықталған балдырлардың көпшілігі әртүрлі суайдындарында кеңінен таралған – космополит түрлер болып саналады [2-5]. Көрсетіліп отырған түрлердің көпшілігі планктондық, аздаған түрлері бентостық түрлерге жатады.

Түйін сөздер: балдырлар, планктон, бентос, систематика, Алакөл көлі.

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БИОРАЗНООБРАЗИЯ ДИАТОМОВЫХ ВОДОРОСЛЕЙ ОЗЕРА АЛАКОЛЬ И ЕЕ СИСТЕМАТИКИ

Аннотация. В Казахстане существует много особо охраняемых природных территории: питомники, национальные парки, заповедники, районы дикой природы, памятники природы, ботанические сады, созданные для сохранения биологических многообразие растений. Во многих из этих областей ученые-флористы

провели научные исследования, связанные синвентаризаций сосудистых растений. Несмотря на существенные интересык изучению флоры, исследование их разнообразие в различных природоохранных сообществах является недостаточным, особенно флоры водоемов. Вводорослив водоемах остаются изученными в незначительной степени.Тем не менее недавно альгологи провели исследование флоры водорослей в особо охраняемых природных территориях различных регионов Казахстана. В статье авторы впервые приводят данные по изучению альгофлоры 15 рек (Урджар, Катынсу, Эмелькуйса, Ыргайты, Жаманты, Жаманутқель, Тасты и т.д.) втекающие в озеро Алакол. Список обнаруженных видов водорослей включает:103 видов, разновидностей и форм, относящиеся к 41 родам, 23 семейству, 13 порядкам, 3 классам и 1 отделу. Составлен конспект и биологическое описание обнаруженных видов водорослей и проведена современная систематика. Большинство видов водорослей, обнаруженные в исследуемых озерах относятся к космополитным формам, широко распространенным в различных типах водоемов [2-5]. Подавляющее большинство обнаруженных видов относятся к планктонным, малая часть видов – бентосные.

Ключевые слова: водоросли, планктон, бентос, систематика, озера Алаколь.

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RICE GRAIN QUALITY FORMATION DEPENDING ON THE MINERAL FERTILIZERS DOSAGES

Abstract. Rice grain quality depends on content and combination of reserve constituents: starch and protein. Hull content, fissuring, shape, and size of grain and hulling easiness are very important for the production of cereals. Taste of cereals, its color, transparency (vitreousness), fast and simultaneous boiling, full content of essential amino acids, vitamins, mineral elements and other nutrients in the grain are of importance for consumers. At the background of treatment with phosphoric and potassic fertilizers (P120K80 kg/ha), application of nitrogen fertilizers (No.160-180 kg/ha) in an optimum dosage, contributes to increase in content of protein, starch and the whole berry in cereals. Increase in the dosage of fertilizers up to N240P180K120 kg/ha did not contribute to increasing of protein and starch content in the grain and the whole berry in cereals; on the contrary, it resulted in a decrease of those parameters and in crop yield.

Key words: rice, grain quality, content of starch, protein and whole berry in the rice grain cereal, effect of increasing dosages of fertilizers on content of the above-specified substances in grain.

The problem of rice production increase is indissolubly tied to the problem of rice grain quality improvement. Thus, improvement of vitreousness just by 1% contributes to the reduction of crushed chips in cereals approximately by 1.2 thousand tons. Improvement of the other quality parameters ensures the additional yield of a thousand ton of rice cereal [1, 2].

Rice grain quality depends on content and combination of reserve constituents: starch and protein, since their share on the basis dry grain substance makes approximately 90%. Multiple agroecological characteristics (level of agricultural methods of cultivation, grain harvesting and processing conditions, etc.) have effect on grain quality as well as on physiological and genetic rice varieties' properties.

Rice grain quality depends on many parameters, their properties and characteristics. For example, hull content; fissuring, shape and size of grain as well as hulling easiness are very important for the production of cereals. Taste of cereals, its color, transparency (vitreousness), fast and simultaneous boiling, full content of essential amino acids, vitamins, mineral elements and other nutrients in the grain are of importance for consumers. According to the rates (8.5 kg per a person) scientifically substantiated by the Institute of Nutrition of the RK National Science Academy, the total quantity of rice available in the Republic of Kazakhstan for domestic consumption is equal to 132.6 thousand tons. Rice cultivating farms of Kyzylorda region and Akdalinskiy area supply rice (mainly short-grain type) to the population of Kazakhstan in sufficient quantity. Nevertheless, over the period of 2007-2012, import of rice (including long-grain type) to Kazakhstan is as follows: from Russia - 57.5%, from China, India and other countries - 28.2%.

Grain shape. According to the IRRI (International Rice Research Institute) scale, by the length-to-width ratio, hulled "yellowish-brown" rice grain is sub-divided into the following categories: narrow, long-grain rice - 3.0; medium length - 2.1-3.0; long, wide - 1.1-2.0; round, short - 1.1 and smaller.

Grain of rice varieties cultivated in Kazakhstan mainly belongs to long and wide and round-shape varieties and length to width ratio is 1.6-2.0 [2.3]. Nevertheless, plant selection breeders are currently breeding long-grain rice varieties. Demand for long-grain varieties in the global market is higher; there-

fore, its price is higher to as compared to the round-grain rice varieties. Rice varieties cultivated in Kazakhstan differ by length to width ratio (i.e. shape) and body of grain and belong to the following types (table 1).

Table 1 – Shape and Different Types of Grain of the Rice Varieties Cultivated in Kazakhstan (according to the information of the Kazakh Rice Cultivation Research Institute)

Shape, Type	Hulled Grain Length to Width Ratio	Sub-types	Grain Body	Rice Varieties Characterizing Particular Features of Types and Sub-types
I	3.5 and higher	–	Vitreous	Lazurny
II	2.8 - 3.4	–	Vitreous	–
III	2.3 - 2.7	1	Vitreous	Solnechny
		2	Half-vitreous	Ushtobe
IV	2.2 and lower	1	Vitreous	Avangard, UzROS 7-13
		2	Half-vitreous	Kuban 3, UzROS 59, Marjan, Karakalpakstan
Note: Kazakhstan bred rice varieties: Marjan, Ushtobe, Altynai, Togusken 1.				

Humidity. Humidity content in the grain determines biochemical and microbiological processes that go on in it. This, to a certain degree, has an effect on technological and food quality of grain. If humidity content in grain exceeds 15-16%, it intensifies microbiological processes resulting in self-heating that changes the chemical composition of grain that becomes more yellowish and loses its cooking and technological properties. Further increase of humidity content in grain even more intensifies the above described unfavorable processes [1-4].

Grain Size and Uniformity. This is connected with the weight of 1,000 grains and determines quality and properties of grain. Rubbing of small, feeble, imperfect grain results in an increase in the number of crushed, fragmented, dust fractions. Hull content of immature, feeble grain increases while its vitreousness degrades. The above-specified grain properties change depending on the level of cultivation technique applied as well as harvesting and processing conditions [1-4].

Hull content is determined by the ratio of hull on bloom and head of grain. Structural-and-mechanical properties of grain and its hull content are interrelated and have certain effect on the cereal output. Therefore, increase or decrease of hull content by 1% results in increase or decrease of cereal output by 1.5-2.0%.

Grain vitreousness. These properties of hulled grain change depending on transparency (vitreousness) and texture of endosperm. Increase of vitreousness improves technological and cooking qualities of cereal. Thus, milling of vitreous grains results in reduction of the amount of small (crushed) admixtures; grains do not stick together while boiling; taste and cooking qualities of porridge and pilaw improve and the marketable appearance of the cereal improves. Vitreousness of the rice grain is within the range of 95-98% [1-4].

Fissuring is inherent to rice grains since the starch content in it is greater than that of protein. Grain fissuring rate depends on changes in temperature and environmental humidity. Changes in temperature and relative humidity of air result also in changes in the grain humidity content. Humidity content of unscoured grain is non-uniform; as a result, different pressure areas are formed in the grain. If such pressure rises above the certain level, a crack appears inside a grain, which cannot be seen from outside [1-4]. Number and size of cracks inside rice grains are different: depending on environmental conditions of rice cultivation, harvesting and processing grain fissuring may vary from 5-10% to 60-70% and there may be one or more cracks in grains. As the result, amount of crushed grains upon its dehulling increases and quantity of the whole-berry grain in cereal decreases. For example, if the grain fissuring rate is above 1-2%, output of the whole-berry grain in cereal upon dehulling will reduce by 0.3-0.8% and crushed admixture amount increases.

Yellow grains appear in the environment of increased humidity and environmental temperature. Thus, if grain turns out to be damp, it will result in self-heating and biochemical processes intensification,

which, in turn, leads to appearance of yellow grains. Total nitrogen, inorganic phosphorus and sugar content in such grains is way higher as compared to usual grain. Such grains' endosperm remains yellow, which downgrades its commercial quality.

Red rice. Seed (episperm) and bran covering (pericarpium) of such grains are of red or reddish-brown color. Upon grain dehulling and milling, some of this coating is removed (destroyed). Nevertheless, red color is preserved in many instances. In order to remove red color together with coating, it is necessary to intensify milling process; this, however, increases the amount of crushed grain and reduces the number of whole grain in cereal. Red seed coat (pericarpium) deteriorates the appearance and decreases market value of rice cereal. For example, if there is 1% of red grains in the finished product, the quality cereal output will be reduced by 0.1%. According to the standard, content of red grains in the made cereals shall not exceed 0.5-2%. Red-grain rice is the persistent weed in many areas including Kazakhstan Aral Sea area. It is similar to the cultivated varieties of rice; its seeds shatter upon maturing and preserve germinating ability in soil within several years [1- 5].

Unripe grain impurities. 1/4 of such grains are not well-filled, endosperm texture is mealy, or only central part is vitreous (transparent). In the harvested rice product (i.e. grain) 1/4 part of unripe caryopsis belongs to black dockage and 1/4 part belongs to grain impurity. If there are unripe grains in the harvested rice, output of the extra, first and second class grain upon dehulling reduces significantly since the amount of crushed and fine-cut fraction increases.

Black dockage. This impurity includes mineral and organic particles (soil lumps, flower scales, beetle particles and remains of other small animals), seeds of wild and cultivated plants, grain impurities (mainly peeled and chopped grains). Such black dockage (weedy impurities) usually is moist; they contribute to self-heating of rice grains and deteriorate their quality.

The structure and yield of the whole kernel is one of the important indicators determining the quality of the cereals. Rice processing into cereals consists in removal of surface layers (hull) at the minimum crushing of caryopsis and preservation of their initial shape. As a result of many agroecological factors, cereals output may be within the range of 60-75%, including the whole kernel output of 60-88%. Subject to strict adherence to the technologies of cultivation, timely harvesting and quality dehulling and processing of grain, recognized varieties of rice in the conditions of the Kazakhstan Aral Sea area yield 60-72% of cereals and 75-88% of the whole kernel. For example, upon dehulling and milling the Marzhan variety grain yields 84-88% of the whole kernel in cereals (2, 3).

Energy Value. Rice kernel energy value is determined based on the amount of released thermal energy from burning 1kg or 1g of grain. Nevertheless, we should determine difference of the released thermal power from grain burning from physiological energy value in the oxidizing processes of organism. The physiological energy value is lower. Dehulling and processing of rice grain also has certain effect on its energy value. Upon dehulling and milling of rice grain, aleuronic layer is removed to a certain degree and digestibility of cereal increases but nutritional value (quality) of it decreases. Grain moisture content also has effect on its energy value. For example, grain moisture content increase from 6.2% to 26.4% results in decrease in energy value thereof from 3963 cal/t to 3566 cal/t [1, 2, 4].

Cooking quality of rice grain is determined by the following parameters: taste, color, consistency of porridge, time of boiling, property of moisture retaining, etc. These parameters change. For example, taste of cooked porridge is assessed as very good, good, satisfactory. Cereal may be of white to brownish color. Porridge consistency varies from loose to sticky and boiling coefficient ranges from 4.3 to 5.1 [1, 2, 4]. Technological, cooking and edibility properties of rice cereals depend on chemical composition of grain. Content (quantity) of rice grain and cereals components are as follows:

Chemical Composition	Dehulled Grain, %	Cereal, %
Starch	76.4-77.5	83.7-85.6
Protein	10.5-12.3	8.5-9.6
Fat	2.6-3.1	0.4-0.5
Fibre	1.2-1.4	0.12-0.14
Ash	1.5-1.6	0.4-0.5

Therefore, high quality rice grain properties shall be as follows: fully ripe, uniform grain size, high vitreousness, low hull content, low and uniform moisture content of grain. At the same time, the content of black dockage, amount of grain and red-grain impurities, the level of contamination with yellowing endosperms should be below or at the same level with the basic standard. Such grain dehulling results in the production of high-quality of rice cereals.

Rice grain quality depending on the area of treatment and dosage of mineral fertilizers. In the course of rice plants growth and development, high quality grain formation depends on mutual influence of the physiological and genetic potential of varieties and agroecological factors (treatment areas, dosages, timing and methods of fertilization and irrigation mode) [1-4]. Since the basic substances ensuring high quality of grain - starch and protein, make 90% of the dry solid matter of the grain. Those substances are intensively accumulated in the optimum photosynthesis process conditions [2, 7].

Treatment with optimum doses of mineral, especially nitrogen fertilizers has a significant effect on the formation of grain quality. Thus, at the background of treatment with phosphoric and potassic fertilizers (P120K80 kg/ha), application of nitrogen fertilizers (No.160-180 kg/ha) in an optimum dosage contributes to increasing in the content of protein, starch and the whole kernel in cereals. Increase in the dosage of fertilizers up to N240P180K120 kg/ha did not contribute to increase of protein and starch content in the grain and the whole kern in cereals; on the contrary, it resulted in a decrease of those parameters (table 2) [2, 7].

Table 2 – Rice Varieties' Grain Quality Depending on the Area of Treatment and Dosage of Mineral Fertilizers

Fertilizer Dosage, kg/ha	Number of Sowed Seeds, pcs/m^2	Plant Density before Harvesting, pcs./m^2	In % per Dry Substance of Dehulled Grain			
			Starch	Protein	Total Nitrogen	Protein Nitrogen
Kuban 3 Variety						
N90P90	700	381	74.3	9.34	1.57	1.31
N180P120	100	55	72.2	10.0	1.68	1.38
	300	180	78.6	11.3	1.90	1.68
	500	281	82.6	12.1	2.03	1.76
	700	405	80.0	11.7	1.97	1.70
	900	471	78.2	10.1	1.69	1.58
Dubovskiy 129 Variety						
N90P90	700	323	71.5	11.0	1.84	1.53
N180P120	100	58	78.2	11.1	1.86	1.62
	300	185	77.1	11.3	1.89	1.72
	500	247	79.8	12.2	2.05	1.80
	700	318	81.4	12.9	2.17	1.94
	900	385	81.4	12.3	2.06	1.82
Marzhan Variety						
N90P90	700	324	81.3	11.6	1.97	1.75
N180P120	100	53	78.8	12.0	2.02	1.82
	300	184	79.1	12.6	2.11	1.93
	500	257	78.2	12.2	2.05	1.84
	700	361	75.1	11.8	2.00	1.81
	900	397	73.5	10.6	1.83	1.61

Consequently, with the increasing dosages, especially of nitrogen fertilizers, there has been a decrease in the yield of rice crops and deterioration of rice grain quality, which means that application of high dosages of fertilizers on rice crops is not profitable from an economic and ecological point of view.

Additionally, this results in environmental pollution (contamination of soil, water in collecting and drainage systems and other water reservoirs).

Starch is a nutritious substance located in the endosperm cells and is the main component of cereal (up to 75-80%). Starch consists of the following two polysaccharides: amylose and amylopectin. Amylose content in the rice grain endosperm varies within the range of 10 to 35%, while that of amylopectin - within the range of 65-90%. Nutritious value of the rice grain is determined by the quantity and amylose to amylopectin ratio. The greater the amylose content in grain is, the more water is consumed and the greater is the grain's volume. Therefore, boiling of grains with medium and high content of amylose does not result in softening and sticking [8] It is important for porridge and pilaw cooking.

Starch content in rice grains is greater than in any other cereal crop. Rice starch is easily digested and its nutritious quality is high. Therefore, by the assimilated energy, the grain of rice has an advantage in comparison with the other cereal crops.

In connection with the physiological and genetic characteristics of rice cultivars and the level of cultivation technology, starch content in the husked grain varies within the range of 65-88%. Application of an optimum dosage (N180P120 kg/ha) of fertilizers results in increase of starch content of the medium-growth, narrow-leaved cultivars Kuban 3, Dubovskiy 129 at the plant population of 180-400 pcs/m^2. The highest starch content (80.0-82.6%) was observed at the plant population in agrophytocenosis of 250-350 pcs./m^2. These crops are highly productive agrophytocenoses of Kuban 3 and Dubovskiy 129 cultivars and the taste of these cultivars' grits turned out to be good (table 2).

Subject to treatment with an optimum dosage (N180P120 kg/ha) of fertilizers, starch content in the Marzhan variety grain did not increase as compared with treatment with the minimum dosage (N90P90 kg/ha) and remained at the same level. In the event of thicker agrophytocenosis (700 and 900 seeds sowed), starch content in the above named cultivars' grain decreased. Correspondingly, in the event of thickened crops (360 to 390 plants/m^2), leaves of the large-leaved varieties of rice samples mutually shade each other deteriorate the photosynthesis process of and, as a result, the synthesis of starch and its transportation to the grain decreases (table 2).

In the course of the grain endosperm development and formation, parenchyma cells filling with carbohydrates (which later turn into starch) comes from the outer, surface cells to the inner cells. If the photosynthesis process deteriorates (depressed) or if grain does not ripe, cavities are formed within the endosperm. Upon dehulling of such grains, kernel is destroyed and large crushed pieces are formed [2].

Protein - the second after starch component contained in large amount in grain (cereals) [1-4]. Reserve rice proteins are mainly represented by prolamins and glutenins that are highly-molecular compounds. Proteins are involved in all vitally important functions and processes in organism. Reserve rice is accumulated in the rice grain endosperm and has effect on sprouts formation upon their germination. Protective proteins play certain role in protection against pathogenic microorganisms. Regulatory proteins activate or suppress molecular-and-biochemical and biological processes in the rice plants, etc. [2, 8]. "Biological effectiveness" of rice grain proteins is due to the high content of essential amino acids and their easy digestion and lower tannin content as compared with the other cereal crops. Nevertheless, total content of protein in the rice grain is less than other crops (table 3). Therefore, increase of the protein content in the rice grain is a positive phenomena from the standpoint of rice breeding.

Study of proteins location in the rice grain endosperm is an important process from the evolution and biochemical point of view [2,9]. Our studies' results show that treatment with a minimum dosage (N90P90 kg/ha) of fertilizers, protein accumulates in the grain of Marzhan and Dubovskiy 129 cultivars longer than in Kuban 3 grain. Treatment with an optimum high dosage (N180P120kg/ha) of fertilizers, protein content in the rice cultivars and specimen increases. Nevertheless, response of different cultivars was different. Treatment with an optimum high dosage (N180P120 kg/ha) of fertilizers, high content of protein in the Kuban 3 cultivar grain was observed at the plant population of 250-280 pcs/m^2 and for Dubovskiy 129 cultivar it was 250-320 plants/m^2 (i.e. after seeding of 300, 500 seeds/m^2). These are the highly productive agrophytocenoses of the above named rice varieties (table 2).

Increase in protein content in the Marzhan cultivar grain was observed at the different plant population (sowed 100, 300, 500, 700 seeds/m^2) in agrophytocenosis (table 2). Therefore, it was found that increase of protein content in rice grain happens depending on the physiological and genetic particular features of cultivars and treatment with an optimum dosage of fertilizers (especially nitrogen ones).

Table 3 – Mean Value of Biological Effectiveness of Proteins of Various Cereal Crops (Y.P. Aleshin, 1993)

Parameter	Dehulled Rice	Wheat	Corn	Barley	Millet	Sorgho
Protein, %	8.5	12.3	11.4	12.8	13.4	9.6
Lysin (g/16g of nitrogen)	8.8	2.3	2.5	3.2	2.7	2.7
Thionine (g/16g of nitrogen)	3.6	2.8	3.2	2.0	3.2	3.3
Methionine (g/16g of nitrogen)	3.9	3.6	3.9	3.9	3.6	2.8
Tryptophan (g/16g of nitrogen)	1.1	1.0	0.6	1.1	1.3	1.0
Pure nitrogen absorbency in protein, %	99.0	96.0	95.0	88.0	93.0	84.8
Biological Effectiveness, %	74.0	55.0	61.0	70.0	60.0	59.2
Specific use of proteins, %	73.8	53.0	58.0	62.0	56.0	50.0
Used protein, % (protein % x specific use, protein/100%)	6.3	6.5	6.6	7.9	7.5	4.8
Tannin content in grain, %	0.1	0.5	0.5	0.8	0.7	1.9

Whole kernel is a valuable part of dehulled and milled rice. The number of whole kernels in rice grain largely depends on the genetic and biological characteristics of varieties, on the level of cultivation technologies, the characteristics of harvesting and processing of grain and other agroecological factors [1, 2, 4, 7].

When applying the minimum dosage (N90P90 kg/ha) of fertilizers, the whole kernel in the grain of the KZROS sample was 82.6%, and that of Marzhan variety was 81.8%. Marzhan variety particular feature consists in formation of a large number of the whole kernel in grain and dehulled rice cereal (84.7-88.5%) at the minimum (N90P90 kg/ha) and opt imum (N180P120 kg/ha) dosages of fertilizers and at various thickness of plant population (100, 300, 500, 700 seeds/m^2) (Table 9.5). Due to this particular feature, Marzhan variety covered 65 to 72% of the area under rice crops of Kyzylorda region.

Table 4 – Technological Properties of Husked Rice Grain Depending on the Area of Treatment and Dosage of Mineral Fertilizers

Fertilizer Dosage, kg/ha	Number of Sowed Seeds, pcs/m^2	Cereal Output and Quality, %			Hull Content, %	Vitreousness, %
		Total	Whole Kernel	Crushed Particles		
Kuban 3 Variety						
N90P90	700	72.0	67.8	32.2	20.0	98
N180P120	100	72.0	68.0	32.0	18.0	97
	300	73.2	68.2	31.8	16.0	98
	500	71.6	84.2	15.8	19.0	98
	700	71.6	78.9	21.1	19.0	98
	900	72.2	75.9	24.1	19.0	97
Dubovskiy 129 Variety						
N90P90	700	72.6	66.4	33.6	16.0	95
N180P120	100	72.4	77.2	22.8	18.0	98
	300	72.2	73.3	26.7	17.0	97
	500	72.4	81.1	18.9	16.0	98
	700	72.0	84.0	16.0	16.0	99
	900	72.4	77.7	22.3	17.0	98
Marzhan Variety						
N90P90	700	71.8	81.8	18.2	17.8	97
N180P120	100	72.0	84.7	15.3	17.7	98
	300	72.1	88.5	12.5	17.5	98
	500	72.0	85.6	14.4	17.5	98
	700	72.4	80.5	19.5	18.1	98
	900	72.3	77.2	21.8	18.6	98

Under the conditions of treatment with an optimum dosage (N180P120 kg/ha) of fertilizers, large number of the whole kernel in the grain of Kuban 3 cultivar was formed at the thickness of plant population of 250-280 plants/m^2, that of Dubovskiy 129 at the plant population of 250-320 plants/m^2. At such a density of plant population, starch and protein content increased in the grain of the above specified cultivars at the above specified plant population. This is plant population of highly effective crops of Kuban 3 and Dubovskiy 129 cultivars. With such agrocenoses, the process of photosynthesis of each plant improves; starch and protein accumulate intensively in the grains. As a result, quality of the above named cultivars' grain improves and taste of cereal improves too. However, starch and protein contend of the heavily thickened and thinned crops did not increase with an increase in the fertilizer dosage and the amount of the whole kernel in the grain and in the range of rice varieties decreased (tables 2 and 4).

The quality of the rice grain and the yield of the whole kernel in the crop depend on the protein and starch content in the grain, since 90% of the dry mass of the grain consists of these named substances. Starch and protein content reduction in grain or change in its ratio contributed to reduction of the whole kernel output and increase in the share of crushed fraction (tables 2 and 4).

Therefore, grain quality formation depends on genetic potential and particular features of rice varieties, on optimum plant population and an intensive photosynthesis process in agrophytocenosis. Agrotechnical measures shall be taken in due time and of proper quality. Correct and timely execution of grain harvesting and processing operation also has a significant effect on grain quality.

Effect of dosages, ratios and methods of mineral fertilizers on quality of rice grain. In the course of rice plants growth and development, high quality grain formation depends on interaction and mutual influence of the physiological and genetic potential of varieties and agroecological factors (treatment areas, dosages, timing and methods of fertilization and irrigation mode, etc.) (table 5) [1, 3, 6, 10].

Table 5 – Effect of Dosages and Ratios of Mineral Fertilizers on Rice Grain Quality (Kuban 3 Variety) [6]

Mineral Fertilizers Dosages and Ratios, kg/ha	Protein, %	Starch, %	Hull Content, %	Weight of 1,000 grains, %	Cereal Output and Quality, %		
					Total	Including	
						Whole Kernel	Crushed Particles
Control (without fertilizers)	7.5	62.2	19.4	31.3	70.2	75.4	24.6
N160	8.1	62.7	20.3	29.8	68.3	70.7	29.3
P120	7.6	61.8	19.3	31.7	71.5	77.9	22.1
K80	7.5	61.5	20.7	31.8	70.3	76.8	23.2
N160P120	8.9	75.6	17.9	31.6	73.6	85.1	14.9
N160K80	8.3	66.3	19.0	31.8	72.3	81.5	18.5
P120K80	7.9	62.7	19.5	31.5	71.2	75.9	24.1
N160P120K80	*9.0*	*76.1*	*18.0*	*31.9*	*74.1*	*89.5*	*11.5*
N80P60K40	7.9	64.8	19.3	30.7	70.8	75.3	24.7
N240P60K40	8.9	71.4	19.8	29.3	69.7	71.3	28.7
N80P180K40	8.0	65.6	19.4	30.8	74.1	76.7	23.3
N80P60K120	8.1	65.4	19.5	31.0	70.8	77.1	22.9
N240P180K80	9.1	69.7	19.8	29.7	68.6	79.4	20.6
N240P60K120	8.9	71.3	19.6	29.3	68.7	73.5	26.5
N80P180K120	8.0	66.7	19.5	30.7	70.3	75.8	24.2
N240P180K120	9.1	68.7	19.7	29.4	67.4	80.3	19.7

Treatment of rice with an optimum dosage (N160-180P120K80 kg/ha) of fertilizers at the nitrogen to phosphorus and potassium fertilizers ratio of 1:0.75:0.5 results in increase of protein and starch content in grain, increase of the whole kernel amount and reduction of hull content thus ensuring the best yield of the highest quality grain. Treatment with the high dosage (N240P180K120 kg/ha) did not result in increase in starch content in grain, whole kernel output but resulted in content of crushed fraction (table 5). Increase

in the dosage, especially that of nitrogen fertilizers, or in the event of reduction of the dosage and change in the ratio of applied fertilizers did not result in increase in the crop yield but resulted in deterioration of grain and cereal quality (table 9.6). Treatment of heavily salinized soil with nitrogen and phosphorous fertilizers in the ratio of N : P = 1 : 1 or 1 : 0.8 resulted in high yield of high quality grain. Nevertheless, it is necessary to determine mobile phosphorous content in soil prior to sowing and it is necessary to take the results into account to determine the ratio of applied mineral fertilizers [1, 3, 6, 10]. Efficiency of various applied nitrogen fertilizers (ammonium sulphate, calurea) turned out similar [3,6,10]. Methods of fertilizers application also have effect on formation of grain quality. Thus, nitrogen fertilizer (calurea) local application resulted in increase in protein content in grain by 0.7% and starch content increased by 4%, in improvement of cereal quality: number of the whole kernels increased by 7% and hull content of grain decreased, weight of 1,000 grains increased (table 6).

Table 6 – Effect of Various Types of Nitrogen Fertilizers and Methods of Their Application on Rice Grain Quality (Kuban 3 Variety) [6]

Types and Dosages of Fertilizers, kg/ha	Protein, %	Starch, %	Hull Content, %	Weight of 1,000 Grains, %	Cereal Output, %		
					Total	Whole Kernel	Crushed Fraction
Types of Nitrogen Fertilizers							
P120K90 (background)	6.6	58.3	20.8	27.4	67.9	62.8	37.2
PK + N_A 120	7.4	64.3	19.6	28.1	69.8	72.0	27.4
PK + N_A 120	7.4	65.0	19.7	28.0	69.6	73.0	27.0
PK + N ($_{A+M}$) 120	7.4	64.7	19.8	27.0	69.5	72.8	27.2
Ways of the Nitrogen Fertilizers Application							
P120K90 (background)	6.6	58.3	20.8	27.4	67.9	62.8	37.2
PK + N_M 120 surface	7.4	65.0	19.7	28.0	69.6	73.0	27.0
PK + N_M 120 local	8.1	69.0	18.6	28.8	71.3	80.0	20.0

Therefore, treatment of the area under rice crops with an optimum dosage of nitrogen fertilizers improves crops structure and grain quality, but application of various forms of nitrogen fertilizers did not have effect on the crop yield and cereal quality. Presence of nitrification inhibitors in the nitrogen fertilizers improved structure of the crops but did not have effect on grain quality. Application of nitrogen fertilizers to the rice crops by local and partial methods improved crop structure, quality and technological parameters.

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МИНЕРАЛЬДЫ ТЫҢАЙТҚЫШТАР ДОЗАСЫНА БАЙЛАНЫСТЫ КҮРІШ ДӘНІ САПАСЫНЫҢ ҚАЛЫПТАСУЫ

Аннотация. Күріш дәні сапасы оның құрамындағы қорлық заттар – крахмал, белок мөлшеріне және арақатнасына байланысты. Жарма (крупа) өндірушілер үшін дәннің қауыздылығы, шытынағыштығы, формасы және ірілігі, оңай ақталуы өте маңызды. Тұтынушылар үшін керегі – жарманың дәмділігі, түсі, шынылығы әрі бір мезгілде пісуі, дән құрамында ауыстырылмайтын амин қышқылдары, витаминдер, минералды элементтер, басқада қоректік заттардың толық болуы. Фосфор және калий тыңайтқыштары фонында (Р120К80 кг/га) азот тыңайтқышын қолайлы дозада (N160-180 кг/га) енгізу жарма құрамындағы белок, крахмал, сынбаған ядро мөлшерін арттырады. Тыңайтқыштар дозасын N240P180K120 кг/га деңгейіне дейін көбейту дән құрамындағы белок, крахмал, сынбаған ядро мөлшерін арттырған жоқ, керісінше азайтты, өнім деңгейіде төмендеді.

Түйін сөздер: күріш, дән сапасы, жарма құрамындағы крахмал, белок, сынбаған ядро мөлшері, тыңайтқыштар дозасының күріш дәні сапасына әсері.

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ФОРМИРОВАНИЕ КАЧЕСТВА ЗЕРНА РИСА В ЗАВИСИМОСТИ ОТ ДОЗ МИНЕРАЛЬНЫХ УДОБРЕНИЙ

Аннотация. Качество зерна риса зависит от количества и сочетания запасных веществ – крахмала и белка. Для производства крупы очень важны пленчатость, трещиноватость, форма и крупность зерна, легкость при обручивании. Для потребителей – вкус крупы, цвет, прозрачность (стекловидность), быстрое и одновременное свариваемость, полное содержание в зерне незаменимых аминокислот, витаминов, минеральных элементов и других питательных ыеществ. На фоне фосфорных и калийных (Р120К80 кг/га) удобрений внесение оптимальной дозы азотных (N160-180 кг/га) удобрений способствует увеличению в зерне белка, крахмала и целого ядра в крупе. Увеличении дозы удобрений до N240P180K120 кг/га не способствовало повышению содержания белка, крахмала в зерне и целого ядра в крупе, а наоборот произошло снижение этих показателей и урожайности.

Ключевые слова: рис, качество зерна, содержание крахмала, белка и целого ядра в крупе зерна риса, влияние возрастающих доз удобрений на содержание выше названных веществ в зерне.

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INFLUENCE OF CHRONIC COMBINED INTOXICATION WITH ZINC, COPPER AND ARSENIC SALTS ON THE CHANGES IN HEMATOLOGIC BLOOD INDICATORS OF RATS

Abstract. This article indicated the investigation results of the chronic combined poisoning effect with zinc, copper and arsenic salts on hematologic blood indicators. Since one of the assessing criteria of the effects of toxic substances on the body is hematological indicators and one of the research objects are indicators of environmental contamination at the organism level, animal experiments have been performed to determine the cytological blood composition. As a result of the conducted investigations, it was revealed that in case of chronic combined intoxication with zinc and copper, copper and arsenic salts, the number of leucocytes increases, and with intoxication with zinc and arsenic salts only the amount of leucocytes decreases. In the first case, there is a development of so-called leucocytosis and leucopenia in the second case. There was also identified an increase in the concentration of erythrocytes, hemoglobin, and mean erythrocyte volume in all experimental groups. Possible causes of changes in morpho-functional blood parameters were revealed and substantiated as well. It was found out that certain groups of heavy metals possess various toxic effects.

Keywords: heavy metals, zinc, copper, arsenic, chronic combined intoxication, blood indicators.

Introduction. Environmental pollution caused by toxic elements emissions, such as heavy metals, is now attracting the interest of researchers all around the world, because toxic elements can accumulate in soil and crops, animal and human organisms [1]. As a result of the rapid development of industrial production, especially in developing countries, the use of heavy metals and synthetic chemicals has rapidly increased. Pollution of the environment with heavy metals creates a danger to human health in particular, as they can bio-accumulate at all levels of the food chain [2]. It is known from scientific studies that heavy metals such as copper, zinc, cadmium, chromium, manganese, lead, arsenic are considered to be toxic to both humans and animals [3].

As for Kazakhstan, pollution of the environment with toxic substances is a topical subject, especially in the areas of mining and processing industry. Akmola region is not an exception. There are mining deposits, where gold, uranium, titanium, iron, manganese, molybdenum are being extracted. One of the largest gold deposits is Vasilkovskoye deposit. The accompanying element in this gold deposit is arsenic, which is why during gold mining process, arsenic enters the environment. Also, soils in Kokshetau area contain such heavy metals as lead, copper, cadmium and zinc [4].

Zinc is considered to be an important element for all life forms, as it is a part of many body's enzymes, it promotes cell growth, takes part in the exchange of proteins, nucleic acids, and vitamin A. Therefore, zinc is of great importance for human health as a trace element [5]. The human body contains 2-3 g of zinc, and it also occurs in muscles, bones, liver, kidneys, lungs, brain, heart and pancreas [6]. 30-40% of zinc is concentrated in the cell nucleus, 50% in the cytosol, and the rest is a part of the cell membranes [5]. Zinc plays a special role in the maintenance of immune functions (cellular and humoral immunity). A lack of zinc can affect congenital and adaptive immunity [7]. Zinc deficiency is associated with acute and

chronic liver disease. Adding zinc to the diet helps protect against toxin-induced liver damage and is used in the treatment of hepatic encephalopathy [8].

However, zinc is considered to be relatively non-toxic to humans [9]. There are three main ways of getting zinc into the body: inhalation (by inhaling air polluted with heavy metals), alimentary (through the gastrointestinal tract during eating), through the skin [10]. Zinc is excreted from the body through the kidneys, skin and intestines [11]. An increase in the concentration of zinc in the body is often associated with copper deficiency. The absorption of copper decreases if there is an increase in the consumption of zinc [12].

Zinc intoxication can cause a decrease in the level of copper, immunity, lipoprotein and copper-containing enzymes. High doses of zinc negatively affect the physiology of urination [13]. They also can inhibit the absorption of copper, sometimes resulting in copper deficiency and, as a consequence, cause anemia. [14] Jerome Nriagu, professor of University of Michigan, has shown that zinc salts are irritating, can cause erosive pharyngitis, gastritis, gastrointestinal bleeding, pancreatitis and also oral, throat, and stomach injuries after swallowing. Prolonged zinc exposure leads to hypoplasia, anemia, leucopenia, and neutropenia, as well as impaired pancreatic function, resulting in increased release of amylase, lipase and alkaline phosphatase into the bloodstream [15].

Copper is an important element for cellular metabolism. It is a cofactor-oxidation-reduction reaction involving intracellular proteins and enzymes such as cytochrome oxidase and superoxide dismutase [16].

Also, copper is a composite component of all soils, an essential element for plants and animals, situated in small amounts in plants and animal organisms, although an increase in copper concentration leads to toxic effects on soil inhabitants. Large doses of copper can cause the destruction of red blood cells and, as a consequence, it can lead to the development of anemia [17]. The main organ, the target, which is struck by chronic copper intoxication firstly, is the liver. Studies conducted on animals have shown that taking large doses of copper can lead to liver and kidney disease. Also, due to the high concentration of copper, hemolytic anemia can be developed. It is caused by acute hepatic necrosis. In some cases, kidney functions may be impaired. As a result of the destruction of liver cells, a large amount of copper enters the bloodstream, damaging the red blood cells [18].

Hepatic and sometimes renal changes are the most common effects found in animals that receive high concentrations of copper. In the organisms of rats, mice, rabbits, chronic copper intoxication leads to mortality increase and retardation growth. In rats, which were subjected to copper sulfate intoxication, there were various cellular cell destruction and membrane disorders, increase in the amount of lysosomes, swelling of mitochondria and tubular microvillus [19].

Arsenic is one of the most toxic metalloids. It can be found in the environment, as a result of natural and anthropogenic influences. It appears in rocks, soil, air, water in small amounts. In nature, arsenic has organic and inorganic forms. Inorganic forms are mainly represented by the form of trivalent metaarsenite (As^{3+}) and pentavalent arsenate (As^{5+}) [20]. Studies of arsenic toxicity show that chronic effects can cause serious impairment of organ functions [21]. Arsenic is a serious carcinogen, intoxication which can lead to the development of malignant tumors, large tumors of the lungs, bladder, and prostate [22]. And also it seriously impacts the cardiovascular system, cause developmental anomalies, neurological and neurobiological disorders, diabetes, hearing loss, hematologic disorders - anemia, leucopenia and eosinophilia [23]. In areas with a high level of arsenic contamination, there was a high death rate from bladder, kidneys, skin and liver cancer [24].

Arsenic and its compounds are excreted from the body with urine and bile and breast milk in small quantities [25]. During the experiment on rats, using chronic arsenic primer, after 12 weeks, arsenic was found in the blood, liver and kidneys [26].

According to Khanturina G.R. date, with chronic zinc, copper and iron salts intoxication, a decrease in the level of leucocytes, erythrocytes, hemoglobin in the blood was revealed [27].

Despite the fact that the influence of heavy metals on the body is well known throughout the world, there is no evidence of the effect of chronic combined intoxication with salts of zinc, copper and arsenic. Therefore, the study of the combined chronic effects of zinc, copper and arsenic salts on the body is of a great interest.

Proceeding from the above mentioned, the purpose of our study was to study the influence of combined chronic intoxication with zinc, copper and arsenic salts on the change in hematological blood indicators of experimental animals.

Materials and methods. The experiments were carried out on 40 white uncontaminated rats. The laboratory rats were divided into four groups for these experiments. They were daily injected intragastrically with solutions of heavy metal salts for three months. The first group (n = 10) consisted of control animals, which were kept under standard conditions, including usual food and water diet. The animals of the second group (n = 10) were injected with solutions of zinc and copper salts, the dose of copper sulfate II was 13 mg/kg, zinc sulfate was 17.5 mg/kg. In the third group (n = 10), there were injected the solutions of copper and arsenic salts, the dose of copper sulfate II was 13 mg/kg, sodium arsenite was mg/kg. Animals of the fourth group (n = 10) were injected with solutions of zinc and arsenic salts, the dose of zinc sulfate was 17.5 mg/kg, sodium arsenite was 1 mg / kg.

Blood sampling is performed three months after the beginning of the experiment. During the experiment, all ethical norms and rules were maintained. Blood was taken from the carotid artery of experimental animals. Hematologic blood indicators were determined on a modern automatic hematological analyzer Nihon Kohden Celltac E (Japan). Blood indicators such as leucocytes, erythrocytes, hemoglobin, hematocrit (HCT), mean erythrocyte volume (MCV), mean hemoglobin in erythrocyte (MCH), mean hemoglobin concentration in erythrocyte (MCHC), ESR using various methods were determined. So, the number of leucocytes was determined with the help of the unified counting method in Goryaev's counting chamber, the erythrocyte concentration was determined by a unified method with 0.9% sodium chloride solution, the hemoglobin level was determined by the hemoglobin cyanide method [28]. The hematocrit was calculated by the formula:

$$\text{HCT}\,(\%) = \frac{RBC\,(109\,MCV\,(fl) * cells/l}{10}$$

The average volume of erythrocytes was calculated according to the formula:

$$\text{MCV} = \frac{Hematocrit\ in\ mcl^3}{\text{Number of erythrocytes in 1 mcl}}$$

The mean hemoglobin content in erythrocyte was calculated by the formula:

$$\text{MCH} = \frac{\text{Hemoglobin in g/l}}{First\ three\ numerals\ of\ erythrocytes\ concentration\ in\ 1l}\,(pg).$$

The mean concentration of hemoglobin in the erythrocyte was calculated by the formula:

$$\text{MCHC} = \frac{Hemoglobin\ in\ g/l}{Hematocrit\ in\ \%} * 100$$

ESR was determined with the help of PR-3 (ESR meter, Panchenkov's apparatus) [29]. The results were processed using *Microsoft Office Excel* software, *Statistica* for Windows. The arithmetical mean (M), the standard error of the arithmetic mean (m) were calculated. The significance of differences in the arithmetic mean was estimated using Student's t-test (t) and significance level (p).

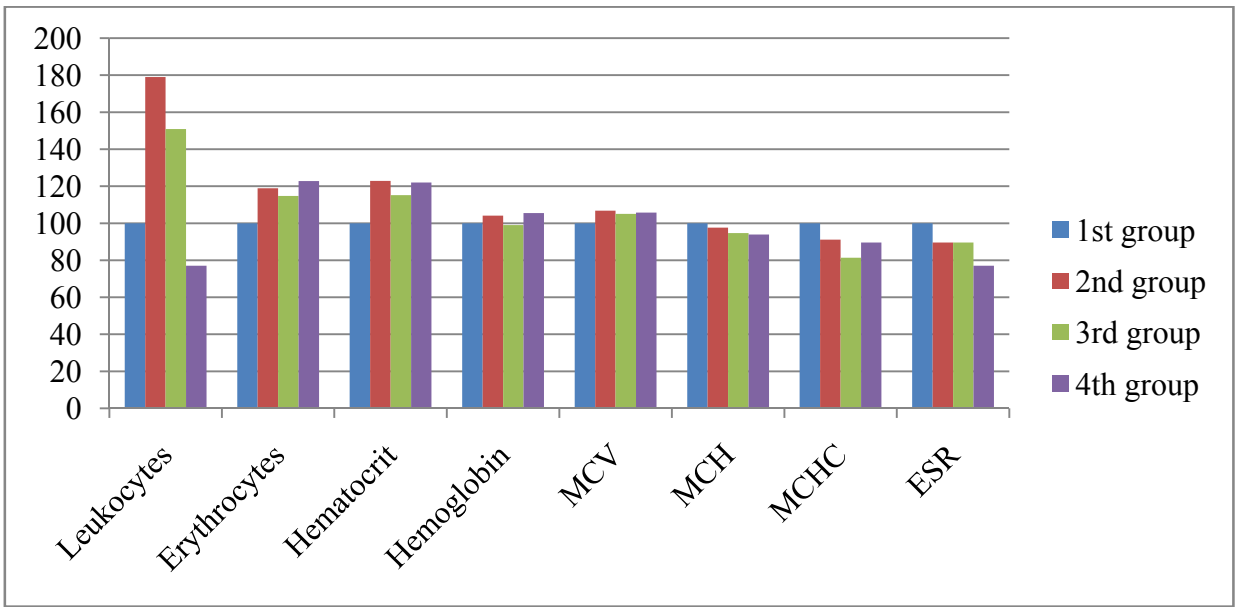
Research results and discussion. The results of our studies show that in case of chronic combined intoxication with zinc, copper and arsenic salts, the number of leucocytes in the blood in the second group increased by 78.89% ($p < 0.05$), in the third group by 50.88% ($p < 0, 05$), and in the fourth group it decreased by 30.94% ($p < 0.05$), in comparison with the control group. Leucocytosis is more marked in the second group (table, figure).

The development of leucocytosis is probably connected with the increased formation of leucocytes in the bone marrow and their release into the bloodstream. It is known from the literature that cytokines are formed in the leucocytes under the impact of toxins in leucocytes, which increase the proliferation and differentiation of leucocytes, as well as the release of the formed elements from the bone marrow. Leucopenia, which is in evidence among the animals of the fourth group, is probably connected with a decrease in the production of leucocytes and their release from the bone marrow into the blood, i.e. there is an oppression of leucopoiesis due to toxic effects on leucopoiesis tissue, as well as increased destruction of leucocytes in peripheral blood and bone marrow.

Hematological indicators of rats' blood with chronic combined intoxication salts of zinc, copper and arsenic salts

Blood indicators	Groups of experimental animals			
	1st group (control group)	2nd group (intoxication with zinc and copper salts)	3rd group (intoxication with copper and arsenic salts)	4th group (intoxication with zinc and arsenic salts)
Leucocytes , $*10^9$/L	6,82±0,11	12,20±0,33*	10,29±0,09*	4,71±0,32*
Erythrocytes,$*10^{12}$/L	7,8±0,16	9,27±0,03*	8,95±0,12*	9,58±0,07*
Hemoglobin, g/L	127±2,97	156,1±1,60*	146,3±2,2*	155±0,61*
Hematocrit, %	47,0±1,13	48,93±0,37	46,49±0,68	49,56±0,36
MCV, fL	49,4±0,64	52,78±0,27*	51,91±0,08**	52,24±0,59**
MCH, pg	17,25±0,04	16,84±0,15	16,33±0,06*	16,2±0,12*
MCHC, g/L	350±5,19	319,1±1,56*	284,8±21,08*	313,5±1,76*
ESR, mm/h	4,8±0,10	4,3±0,11**	4,3±0,11**	3,7±0,11*

*The differences are significant compared to the control group, with p <0.05; ** - the differences are significant in comparison with the control group, with p <0.01, n - the number of animals in the groups.



Dynamics of hematological indicators in chronic combined intoxication with salts of zinc, copper and arsenic

There were also changes in the leukocyte formula. The number of lymphocytes decreased in the second group by 14.42% (p <0.05), in the third group by 19.36% (p <0.05), in comparison with the control group. In the fourth group, the results were practically equal to the control group. The number of monocytes increased in the second group by 220% (p <0.05), in the third group by 155% (p <0.05), in the fourth group by 61.18% (p <0.05) in comparison with the control group of animals. The number of segmented neutrophils increased in the second group by 26.5% (p <0.05), in the third group by 83.13% (p <0.05), in the fourth group by 37.35% (p <0.05), in contrast to the control data.

An increase in red blood cells was observed in all experimental groups. In the second group it was 18.85% (p <0.05), in the third group was14.74% (p <0.05), in the fourth group was 22, 8% (p <0.05). At the same time, the amount of hemoglobin also increased in the second group by 22.9% (p <0.05), in the third group by 15.20% (p <0.05), in the fourth group by 22% (p <0,05), in comparison with the results of the control group. It can be assumed that this increase in the number of erythrocytes is connected with a thickening of the blood due to excessive loss of body fluid, or increased formation of erythrocytes in the bone marrow as a result of oxygen deficiency. Perhaps, this can be explained by the hyperproduction of

erythropoietin in the defeat of kidney tissue and the resolution of the liver parenchyma. It is well known that erythrocytosis lead to an increase in blood viscosity, aggregation of uniform elements, microcirculation and the appearance of dystrophic changes in organs and tissues.

The parameters of the hematocrit remained within the norm, in comparison with the control data. So, the value in the second group increased by 4.11%, in the third group it decreased by 1%, in the fourth group it increased by 5.45%.

The mean erythrocyte volume increased in the second group by 6.8% ($p < 0.05$), in the third group by 5.08% ($p < 0.01$), in the fourth group by 5.75% ($p < 0.01$), although the mean hemoglobin concentration in the erythrocyte decreased in the second group by 2.38% ($p < 0.05$), in the third group by 5.33% ($p < 0.05$), in the fourth group by 6.09% ($p < 0.05$). An increase of this indicator may show the development of liver diseases, as well as a violation of bone marrow activity in severe leucocytosis, which is confirmed by the results of our studies.

The mean hemoglobin content in the erythrocyte decreased in the second group to 8.83% ($p < 0.05$), in the third group to 18.63% ($p < 0.05$), in the fourth group by 10.43% ($p < 0, 05$). Also, the mean and hemoglobin concentration in the erythrocyte in all the experimental groups decreased, in the second group to 2.38% ($p < 0.05$), in the third group to 5.33% ($p < 0.05$), in the fourth group to 6, 09% ($p < 0.05$) compared with the control data. This is possibly due to the development of absolute hypochromia of erythrocytes, as a result of a violation of iron assimilation.

Erythrocyte sedimentation rate decreased in the second group to 10.42% ($p < 0.01$), in the third group to 10% ($p < 0.01$), in the fourth group to 22.92% ($p < 0.05$). Decrease of ESR is probably due to a violation of protein synthesis in liver failure, as well as the violation of the columns formation, which is caused by the change in the form of red blood cells.

Conclusions. Thus, chronic combined intoxication of experimental animals with zinc, copper and arsenic salts led to the significant deviations from the norm of hematological parameters. In this case, the development of neutrophilic leucocytosis in groups of combined effect of zinc and copper, copper and arsenic salts, as a manifestation of poisoning, was observed. The revealed monocytosis in our experiments is the immune reaction of the organism to the action of heavy metals. Also, leucopenia in the group of combined intoxication with zinc and arsenic salts was detected. Because the number of erythrocytes increased, as well as their volume, this led to an increase in hemoglobin and may also indicate a violation of liver function. The increase in erythrocytes can also be connected with the activation of erythropoiesis with the increased erythropoietin formation, which is probably caused by a lack of oxygen, possible neoplasm in the kidneys and adrenal glands.

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МЫРЫШ, МЫС ЖӘНЕ МЫШЬЯК ТҰЗДАРЫНЫҢ ГЕМАТОЛОГИЯЛЫҚ КӨРСЕТКІШРІНІҢ ӨЗГЕРІСІНЕ ҚОСАРЛАСА СОЗЫЛМАЛЫ ӘСЕРІ

Аннотация. Осы зерттеуде мырыш, мыс және мышьяк тұздарымен қосарласа созылмалы әсері нәтижелерінде гематологиялық көрсеткіштердің өзгерістері берілді. Ағзаға улы заттар әрекеттерін бағалау өлшемдерінің бірі – гематологиялық көрсеткіштерді талдау болып саналады. Жүргізілген зерттеулер нәтижесінде мырыш пен мыс, мыс және мышьяк тұздарымен созылмалы қосарласқан улану кезінде лейкоциттер саны көбейіп, ал, мырыш және мышьяк тұздарымен улануы кезінде лейкоциттер саны төмендегені анықталды. Бірінші жағдайда лейкоцитоз және екінші жағдайда лейкопения дамиды. Сондай-ақ, барлық эксперименттік топтарда эритроциттердің, гемоглобиннің, эритроциттердің орташа көлемінің артуы көрсетіледі. Мақалада қан көрсеткіштерінің морфо-функционалдық өзгерістері ықтимал себептері анықталған және негізделген. Бұл ауыр металдар белгілі бір топтары әр түрлі іс-әрекеттерімен ерекшеленетінің көрсетеді.

Түйін сөздер: ауыр металдар, мырыш, мыс, мышьяк, қосарласа созылмалы улану, қан көрсеткіштері.

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ВЛИЯНИЕ ХРОНИЧЕСКОЙ СОЧЕТАННОЙ ИНТОКСИКАЦИИ СОЛЯМИ ЦИНКА, МЕДИ И МЫШЬЯКА НА ИЗМЕНЕНИЕ ГЕМАТОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ КРОВИ КРЫС

Аннотация. В статье представлены результаты исследования влияния хронических сочетанных отравлений солями цинка, меди и мышьяка на гематологические показатели крови. Поскольку одним из критериев оценки действия токсичных веществ на организм являются гематологические показатели и одним из объектов исследования, – индикаторы загрязнения окружающей среды на организменном уровне, были проведены эксперименты на животных с определением цитологического состава крови. В результате проведенных исследований выявлено, что при хронической сочетанной интоксикации солями цинка и меди, меди и мышьяка увеличивается количество лейкоцитов, а при интоксикации солями цинка и мышьяка снижается. Развиваются так называемые, лейкоцитоз – в первом случае и лейкопения – во втором. Также обнаружено увеличение содержания эритроцитов, гемоглобина, среднего объема эритроцитов во всех экспериментальных группах. Выявлены и обоснованы возможные причины изменений морфо-функциональных показателей крови. Выяснено, что определенные группы тяжелых металлов обладают различными токсическими действиями.

Ключевые слова: тяжелые металлы, цинк, медь, мышьяк, хроническая сочетанная интоксикация, показатели крови

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MODELLING THE PROBABILITY OF EMERGENCE OF MULTIPLE DRUG RESISTANCE IN INFLUENZA VIRUS

Abstract. Antiviral drug resistance in influenza virus poses a serious threat to public health, particularly during the epidemic period. In this article we evaluated the probability of emergence of multiple drug resistance to adamantane and the oseltamivir in influenza virus. 35,061 amino acid sequences of both the neuraminidase and M2 matrix protein genes were selected for analysis in the international NCBI database. In order to search for and count the sites responsible for the formation of susceptibility to antiviral drugs, the scripts were developed and written in the Python 3.6 programming language. Evaluation of the possibility of simultaneous existence of amino acid substitutions in the M2 and NA genes leading to the formation of resistance showed that these paired mutations are antagonistic to each other, and theoretically the occurrence of such virus strains is unlikely. The findings can serve as a basis for the practical application of complex therapy with the drugs based on adamantane derivatives and neuraminidase inhibitors against influenza virus.

Key words: influenza virus, antiviral resistance, amino acid substitutions, fitness cost, genetic linkage.

Introduction. Evolution of antibiotic resistance is usually the result of small changes that allow the microbes or other organisms to survive under special circumstances when the organism encounters an extremely strong selection pressure due to the presence of any antibiotic drug. In other cases, this is the result of the transfer of pre-existing antibiotic resistance genes from one microbe to another and selection of such microorganisms in an antibiotic-containing medium. Even in the first example, evolution does not create a truly new function. Such changes often make microorganisms less adapted to normal growth conditions – their efficiency declines, manifesting itself in reduced virulence, transmission, and growth rate, while these mutants are able to survive treatment with antibiotics. This effect is widely recognized and called the fitness cost of antibiotic resistance or “fitness cost”.

Energy costs of drug resistance are real, and biological realities such as “fitness cost” and other limitations of the evolution of microorganisms play a vital role in shaping strategies used to combat resistance to antibiotics, antiviral resistance, etc. In fact, if it weren’t for the “fitness cost”, in many cases drug-resistant bacteria and viruses would multiply without restriction, and soon replace susceptible strains. However, in practice, because of the “fitness cost”, resistant strains are replaced by susceptible strains when the drug is removed from the medium, and the selection pressure is weakened. Thereby, the susceptible strain will eventually defeat the resistant one in a drug-free medium. It can take several days, or several decades, depending on the relative difference in the “fitness cost”. The difference in the “fitness cost” between susceptible and resistant strains can also be leveled by compensatory mutations, but will never be zero [1, 2].

A number of drug resistance mutations are incompatible with each other because of the cumulative effect of the associated negative effects on the body, as well as in relation to the need to maintain a certain general genomic context for the states of many other polymorphic alleles in the genome, including compensatory mutations.

There is a large number of works devoted to the study of the "fitness cost" concept in various bacteria. However, there are very few similar studies on viral infection models. In this article, we have tried to evaluate the probability of emergence of multiple drug resistance to adamantane and oseltamivir in influenza virus.

There are currently two classes of the most common antiviral drugs used in the treatment of the influenza virus infection: adamantane derivatives (amantadine, rimantadine) and neuraminidase inhibitors (oseltamivir) [3].

The study into the genetic basis of resistance showed that all rimantadine-resistant strains have mutations in the transmembrane domain of the M2 protein, namely at positions 26, 27, 30, 31, and 34 [4, 5]. In this case, a structure of the mutant transmembrane domain of the M2 protein changes, which leads to a change in the structure of the viral ion channel [6, 7].

The frequency of appearance of a virus resistant to neuraminidase inhibitors remains low compared to the resistance to adamantanes. However, every year the share of oseltamivir-resistant variants increases throughout the world [8]. Genetic studies of influenza virus strains have revealed a histidine to tyrosine amino acid substitution (H274Y mutation) in the neuraminidase protein, leading to the formation of resistance to the oseltamivir.

Therapy for the disease with several drugs that act at different stages of the viral life cycle is now considered to be one of the most effective approaches. And, perhaps, the complex therapy may reduce the likelihood that any single mutation will lead to the emergence of resistance.

Materials and methods. *Objects of the study.* The amino acid sequences of the neuraminidase protein and matrix protein M2 of the influenza A virus, obtained from the international NCBI database [9], were used in this study. Information on the frequencies of amino acid substitutions in 53,761 genomes of the influenza virus was analyzed. The data were used to examine the dynamics of mutation accumulation in the global population of the influenza virus.

Search and analysis of amino acid substitutions leading to the emergence of resistant virus strains. The search for mutations responsible for the formation of resistance to adamantane in the M2 protein gene was carried out at positions 26, 27, 30, 31, and 34, initiating at the first start codon.

The search for mutations responsible for the formation of resistance to the oseltamivir in the neuraminidase protein was carried out at position -2 from the location of the EEC/SSC/RY/H/F pattern.

The mutations associated with drug resistance were established according to the NCBI database records [9].

To determine the conservative pattern in the neuraminidase gene, multiple amino acid sequence alignment was performed using the MEGA 7 [10] and Lasergen software (version 12, DNASTAR, Inc, Madison, WI).

Determination of mutation frequencies. The frequencies of single mutations responsible for the formation of mono resistance have been calculated. The mutation frequency was calculated as the ratio of the number of each mutation to the total number of amino acid sequences according to formula (1):

$$f = n/N, \qquad (1)$$

where f – mutation frequency, n – number of mutations, N – total number of amino acid sequences.

To analyze the character of amino acid substitutions, the Grantham's distance was used (figure).

With a physicochemical distance above 57.9, the substitution is assumed to be conservative, or radical in the opposite case [11].

Calculation of linkage disequilibrium (LD) parameter for the pairs of mutations. For each pair of mutations, a parameter LD, characterizing the disequilibrium concatenation of the signs, was calculated by the formulae (2)-(4) [12]:

$$LD = \sum_{i=1}^{k} \sum_{j=1}^{l} p(A_i) p(B_j) \times \left| \frac{D_{ij}}{D_{ij}^{\max}} \right|, \qquad (2)$$

$$D_{ij} = p(A_i B_j) - p(A_i) p(B_j), \qquad (3)$$

$$D_{ij}^{\max} = \begin{cases} \min\left[p(A_i)p(B_j),(1-p(A_i))(1-p(B_j))\right] & D_{ij} < 0 \\ \min\left[p(A_i)(1-p(B_j)),(1-p(A_i))p(B_j)\right] & D_{ij} \geq 0 \end{cases}, \quad (4)$$

where *pA* and *pB* are the allele frequencies at loci *A* and *B*, *pAB* is the frequency of gametes carrying a pair of alleles A and B at two loci.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
A	100	9	41	50	45	73	59	54	50	54	59	45	86	54	45	54	73	68	32	45
C		100	27	23	4	27	18	9	4	9	9	36	23	27	18	45	32	9	0	9
D			100	77	18	54	59	23	50	18	27	86	50	68	54	68	59	27	14	27
E				100	36	54	82	36	73	36	41	77	54	86	73	64	68	41	27	41
F					100	27	54	86	50	86	86	27	45	45	54	27	50	77	82	86
G						100	54	36	41	36	41	64	77	59	41	73	73	50	14	32
H							100	54	82	54	59	68	64	86	86	59	77	59	45	59
I								100	50	95	95	32	54	50	54	32	59	86	68	82
K									100	50	54	54	50	73	86	41	64	54	50	59
L										100	91	27	54	45	50	32	54	82	68	82
M											100	32	59	50	54	36	59	86	68	82
N												100	54	77	59	77	68	36	18	32
P													100	64	50	64	82	68	32	50
Q														100	77	68	77	54	41	54
R															100	50	64	54	50	64
S																100	73	41	18	32
T																	100	68	41	54
V																		100	59	73
W																			100	82
Y																				100

Grantham's physicochemical distance matrix for amino acid substitutions

Bioinformatics calculations. To search for amino acid substitutions in the examined genes responsible for the emergence of drug resistance and calculate frequencies of both mono- and paired mutations, we have used own scripts written in the Python 3.6.

Results. *Finding whole genome amino acid sequences of influenza A virus from the NCBI database.* The whole genome amino acid sequences of the NA and M2 proteins were obtained from the NCBI database to carry out an analysis. The appropriate filters were used to collect relevant data, which make it possible to get rid of laboratory isolates, mixed strains, and duplicating sequences.

In total, 53,761 whole genome sequences of the NA and M2 genes of the influenza A virus circulating in the world between 1902 and 2017 were obtained from the database. In view of the fact that the strains are often deposited in the NCBI without the first start codon, or, conversely, have an additional sequence before it, the sequences obtained were optimized in the subsequent experiments. Using own scripts written in Python 3.6, all sequences of the M2 and NA proteins were aligned with the first start codon. In addition, the strains, which simultaneously included genome sequences of the M2 and NA proteins, meeting the requirements as described above, were sorted.

As a result of all manipulations, 35,061 amino acid sequences of both the neuraminidase and M2 matrix protein genes were selected for further analysis.

Analysis of amino acid substitutions leading to the emergence of resistant strains of influenza virus. Using the obtained amino acid sequences, the search and counting of sites responsible for the formation of resistance to adamantane and to the oseltamivir were carried out in subsequent experiments. In view of the fact that, despite the optimization, the amount of data analyzed was extremely large, direct search and counting of sites were not possible. At the same time, the existing software (both commercial and free) did not allow to carry out the required manipulations. We have developed and written own scripts in the Python 3.6, which enables us to perform a search and counting of sites for the given parameters quickly and optimally.

According to the published data, the formation of resistance to adamantane causes by amino acid substitutions in the matrix protein of the influenza virus at positions 26, 27, 30, 31, and 34 relative to the start codon. All amino acid substitutions in the M2 protein and their absolute number are shown in table 1.

Table 1 – Amino acid substitutions in the M2 protein gene

Substitution	Number	Phenotype	Substitution	Number	Phenotype
26A	1	n/d	30A	35007	S
26F	51	R	30E	1	n/d
26I	346	R	30I	11	n/d
26L	34645	S	30N	1	n/d
26N	3	n/d	30S	22	n/d
26P	1	n/d	30T	16	R
26R	1	n/d	30V	3	R
26S	8	n/d	31K	1	n/d
26V	5	n/d	31A	1	n/d
A27	1144	R	31R	2	n/d
27E	1	n/d	31I	6	n/d
27F	38	n/d	31N	20579	R
27G	20	n/d	31G	4	n/d
27I	1585	R	31L	11	n/d
27L	5	n/d	31D	7	n/d
27M	3	n/d	31S	14450	S
27S	2	R	34G	35039	S
27T	345	R	34I	15	n/d
27V	31918	S	34E	3	R
			34W	4	n/d

Notes: S – susceptibility phenotype; R – resistance phenotype; n/d – no data.

However, during the analysis, a number of amino acid substitutions were recorded in the matrix protein gene, which, according to the literature data, did not clearly differentiate into mutations leading to susceptibility or resistance, but nonetheless affecting the phenotype to some extent.

In order to take into account the influence of these substitutions on the formation of resistance in influenza virus and calculate the linkage disequilibrium parameter, an analysis was made to determine the character of the amino acid substitutions for the M2 protein. To determine the nature of the mutations, which were not described in the literature, the Grantham's physicochemical distance matrix was used. The results are shown in table 2.

In addition to the mutation at position 274 resulting in resistance to oseltamivir, a number of substitutions are known for the neuraminidase protein. However, some of them are related to compensatory mutations, while the other part is specific only for certain genetic groups of the influenza virus and cannot provide an adequate picture of resistance formation. As a result, only an amino acid substitution at position 274 in the neuraminidase protein was used in the final analysis. However, in view of the fact that the neuraminidase protein sequences differ in length due to the presence of insertions/deletions that are responsible for pathogenicity, the search for position 274, initiating at the start codon, will not provide adequate amino acid content in this site. Therefore, in preliminary studies, a conservative pattern was searched for, which would be located in the immediate vicinity of the necessary site.

The search for a conservative pattern was carried out using the MEGA 7 and Lasergen 12 software. A rather conservative EEC/SSC/RY/H/F pattern, located two amino acids after the site responsible for the formation of resistance to the oseltamivir, was found.

Using the found pattern and own scripts, the number of sites responsible for the formation of susceptibility/resistance was determined (table 3).

Table 2 – Determination of the character of amino acid substitutions in the M2 protein gene

Amino acid substitution	Number of substitutions	Character of substitutions
26A	1	R
26N	3	R
26P	1	R
26R	1	R
26S	8	R
26V	5	S
27E	1	R
27F	38	S
27G	20	R
27L	5	S
27M	3	S
30E	1	R
30I	11	R
30N	1	R
30S	22	R
31K	1	R
31A	1	R
31R	2	R
31I	6	R
31G	4	S
31L	11	R
31D	7	S
34I	15	R
34W	4	R
Notes: S – susceptibility phenotype; R – resistance phenotype.		

Table 3 – Amino acid substitutions in the neuraminidase protein gene

Substitution	Number of substitutions	Phenotype
274H	34615	S
274Y	446	R
Notes: S – susceptibility phenotype; R – resistance phenotype.		

In subsequent experiments, in order to calculate the linkage disequilibrium parameter, it was required to determine a possibility of the simultaneous existence of amino acid substitutions in the M2 and NA genes leading to the formation of resistance within the same organism. Therefore, only mutations leading to the resistance phenotype were used for the analysis. In other words, the frequencies of mutations, both described in the literature and calculated according to the Grantham's distance, were summed up within the same site. Based on the values obtained, the frequencies of mutations responsible for resistance to adamantanes at positions 26, 27, 30, 31, and 34 for protein M2 and to oseltamivir at position 274 for the neuraminidase protein were calculated. The values of theoretical and practical frequencies of paired mutations were further calculated. Practical frequencies were obtained on the basis of direct counting of substitutions in the M2 and NA genes (table 4).

Using the obtained frequency values, the linkage disequilibrium (LD) parameter was calculated for paired mutations.

Table 4 – Frequencies of paired mutations

Paired mutations	Practical values	Theoretical values
26-274	0,0	1,50E-04
27-274	1,7E-04	1,12E-03
30-274	0,0	1,96E-05
31-274	3,68E-03	7,47E-03
34-274	0,0	7,98E-06

The linkage disequilibrium (LD) parameter is a non-random distribution of allele frequencies at different loci, which can be due not only to the close genetic linkage but also to the adaptive advantage of the particular combination of alleles, whose frequency accordingly increases in comparison with the frequency expected with a random distribution.

The LD parameter can take both positive and negative values. Positive linkage values indicate that two loci occur together in the same haplotype more often than expected, while negative LD exists when alleles occur together in the same haplotype less often than expected. The data are shown in table 5.

Table 5 – Linkage disequilibrium parameter for paired mutations in the M2 and NA proteins

Paired mutations	LD value
26-274	-1,50E-04
27-274	-9,70E-04
30-274	-2,00E-05
31-274	-3,95E-03
34-274	-8,16E-06
LD_{common}	-5,12E-03

The resulting negative LD values indicate that these paired mutations are antagonistic to each other. The common LD parameter also has a negative value. Theoretically, the emergence of influenza virus strain, which simultaneously possesses such pairs of amino acid substitutions in the NA and M2 genes, is extremely unlikely. However, the LD parameter has a low negative value, tending to zero, which in turn may indicate a lack of analyzed data or a relative general neutrality of the simultaneous presence of two mutations resulting in the formation of multiple drug resistance.

Conclusions. The concept of energy relevance is a characteristic of any living organism. The acquisition or loss of structures, including genetic ones, inevitably leads to a change in the energy status of the organism. Restoring this balance entails changes in the structure of the genome or epigenome. However, this can be problematic for viruses due to the limited size of their genome and lead to irreversible fatal consequences.

Based on the data presented in the NCBI database, as well as the analysis carried out, it was suggested that resistance to adamantane and neuraminidase inhibitors (oseltamivir) cannot exist simultaneously due to the energy irrelevance. In this article, the effect of compensatory mutations on the decrease of the “fitness cost” was not taken into account; however, the findings can serve as a basis for subsequent studies.

Theoretical calculations obtained in the framework of this work undoubtedly require a comprehensive experimental verification and evaluation. However, if the results are confirmed, the data may serve as a basis for reviewing or clarifying existing regimens for influenza virus treatment.

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ГРИПП ВИРУСЫНДАҒЫ КӨПТЕГЕН ДӘРІГЕ ТҰРАҚТЫЛЫҚТЫҢ ПАЙДА БОЛУ МҮМКІНДІГІН МОДЕЛДЕУ

Аннотация. Тұмау вирусының дәрілік заттарға қарсы вирусқа қарсы тұрақтылығы қоғамдық денсаулық үшін, әсіресе эпидемия кезеңінде елеулі қатер болып табылады. Осы мақалада біз грипп вирусындағы адамантандық қатардағы препараттар мен осельтамивир препаратына кұптеген дәрілік тұрақтылығының пайда болу мүмкіндігін бағаладық. Талдау үшін NCBI халықаралық базасында нейраминидаз және М2 матрикстік ақуыз ретінде гендердің 35061 аминқышқылдарының бірізділігі бойынша іріктедік. Вирусқа қарсы препараттарға сезімталдықты қалыптастыруға жауапты сайттарды іздеу және санау үшін Python 3.6 бағдарламалау тілінде скрипттер әзірленіп, жазылды.

Тұрақтылықты қалыптастыруға әкелетін М2 және НА гендеріндегі амин қышқылының алмастыруларының бір мезгілде болу мүмкіндігін бағалау бұл мутациялардың жұптары бір-біріне антагонист болып табылады және теориялық түрде мұндай штаммдардың пайда болуы екіталай.

Алынған нәтижелер грипп вирусына қатысты нейраминидаз ингибиторлары және адамантан туындылары препараттарымен кешенді терапияны практикалық қолдану үшін негіз бола алады.

Түйін сөздер: грипп вирусы, вирусқа қарсы тұрақтылық, аминқышқылдық алмасулар, fitness cost, тіркеспе гендер.

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МОДЕЛИРОВАНИЕ ВОЗМОЖНОСТИ ВОЗНИКНОВЕНИЯ МНОЖЕСТВЕННОЙ ЛЕКАРСТВЕННОЙ УСТОЙЧИВОСТИ У ВИРУСА ГРИППА

Аннотация. Антивирусная резистентность вируса гриппа к лекарственным препаратам является серьезной угрозой для общественного здравоохранения, особенно в эпидемический период. В этой статье мы провели оценку возможности возникновения множественной лекарственной устойчивости к препаратам адамантанового ряда и препарату осельтамивир у вируса гриппа. Для анализа было отобрано по 35061 аминокислотных последовательностей генов как нейраминидазы, так и матриксного белка M2 в международной базе NCBI. Для поиска и подсчета сайтов, отвечающих за формирование чувствительности к противовирусным препаратам, были разработаны и написаны скрипты на языке программирования Python 3.6. Оценка возможность одновременного существования аминокислотных замен в генах M2 и NA, ведущих к формированию устойчивости показала, что данные пары мутаций являются антагонистическими по отношению друг к другу, и теоретически возникновение таких штаммов вируса маловероятно. Полученные результаты могут служить основанием для практического применения комплексной терапии препаратами производных адамантана и ингибиторами нейраминидазы в отношении вируса гриппа.

Ключевые слова: вирус гриппа, противовирусная резистентность, аминокислотные замены, fitness cost, сцепленность генов

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CO-CIRCULATION OF INFLUENZA A AND B VIRUSES AMONG HUMANS IN THE ARAL REGION OF THE REPUBLIC OF KAZAKHSTAN DURING THE 2015–2017 EPIDEMIC SEASONS

Abstract. In 2015-2017, 2105 biosamples (1978 nasopharyngeal swabs and 127 serums) were obtained from patients in polyclinics and infectious diseases hospitals in Aktobe and Kyzylorda regions of the Republic of Kazakhstan.

Using the polymerase chain reaction for 1978 samples collected from humans, the genetic material of the influenza A virus was detected in 10.86% of cases, that of the influenza B virus in 9.15%. While subtyping influenza A virus RNA, A/H1 subtype was identified in 9.76% of samples, A/H3 subtype in 89.30%. The results obtained from the screening of nasopharyngeal swabs in the polymerase chain reaction, as well as serological data in the hemagglutination inhibition reaction and enzyme immunoassay indicate co-circulation of the A/H1N1, A/H3N2 and B influenza viruses in humans in the Aktobe and Kyzylorda regions of the Republic of Kazakhstan during the 2015-2017 epidemic seasons.

In the virological study of nasopharyngeal swabs obtained from humans, 13 hemagglutination agents were isolated on chick embryos, 10 of which were identified in the hemagglutination inhibition and neuraminidase inhibition assays as influenza A/H1N1 viruses, and 3 as influenza B viruses.

The results from virological and serological studies indicate the need for continuous surveillance of the influenza virus circulation among humans in Aktobe and Kyzylorda regions in order to timely predict epidemic outbreaks and carry out preventive measures.

Keywords: circulation, influenza virus, subtype, isolate, hemagglutinin, neuraminidase, chain polymerase reaction, enzyme-linked immunosorbent assay.

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СОЦИРКУЛЯЦИЯ ВИРУСОВ ГРИППА А И В СРЕДИ ЛЮДЕЙ В АРАЛЬСКОМ РЕГИОНЕ РЕСПУБЛИКИ КАЗАХСТАН В ЭПИДЕМИЧЕСКИЕ СЕЗОНЫ 2015-2017 ГГ.

Аннотация. В 2015-2017 гг. в Актюбинской и Кызылординской областях РК от больных людей в поликлиниках и инфекционных больницах получено 2105 биопроб (1978 носоглоточных смыва и 127 сывороток крови).

В полимеразной цепной реакции в 1978 образцах, собранных от людей, генетический материал вируса гриппа А был обнаружен в 10,86% случаев, вируса гриппа В – в 9,15%. При субтипировании РНК вируса гриппа А подтип А/Н1 идентифицирован в 9,76% проб, А/Н3 – в 89,30%.

Результаты, полученные при скрининге носоглоточных смывов в полимеразной цепной реакции, также как и данные серологических исследований в реакции торможения гемагглютинации и иммуноферментном анализе, указывают на социркуляцию вирусов гриппа A/H1N1, A/H3N2 и В у людей в Актюбинской и Кызылординской областях РК в эпидемические сезоны 2015-2017 гг.

При вирусологическом исследовании носоглоточных смывов, полученных от людей, на куриных эмбрионах выделено 13 гемагглютинирующих агентов, 10 из которых идентифицированы в реакции торможения гемагглютинации и реакции ингибиции нейраминидазной активности как вирусы гриппа A/H1N1, три - как вирусы гриппа В.

Результаты вирусологических и серологических исследований свидетельствуют о необходимости проведения постоянного надзора за циркуляцией возбудителей гриппа среди людей в Актюбинской и Кызылординской областях с целью своевременного прогнозирования эпидемических вспышек и проведения профилактических мероприятий.

Ключевые слова: циркуляция, вирус гриппа, подтип, изолят, гемагглютинин, нейраминидаза, цепная полимеразная реакция, иммунноферметный анализ.

Введение. Из острых респираторных вирусных инфекций наибольшее клиническое и эпидемиологическое значение для людей имеет грипп. Ежегодно во всем мире регистрируется около 600 миллионов случаев гриппа, причем 3 миллиона человек страдают серьезными заболеваниями, которые приводят к летальному исходу в 250 000 - 500 000 случаев [1].

С 1890 г. вирусы гриппа типа А периодически с интервалом в 10-40 лет вызывают пандемии, обусловленные появлением радикально новых вариантов вирусов гриппа, против которых в человеческой популяции почти или совсем нет иммунитета – процесс, называемый антигенным шифтом. Последняя пандемия гриппа 2009/2010 г. была вызвана вирусом A(H1N1)pdm09, содержащим сложную комбинацию сегментов генов вирусов свиного, птичьего и человеческого гриппа. Этот вирус полностью заменил циркулировавшие ранее сезонные вирусы A(H1N1) и продолжает циркулировать во всем мире вместе с вирусами А (H3N2) и типа В [2].

Вирусы гриппа являются наиболее изменчивыми из вирусов человека благодаря высокой скорости мутаций, быстрой репликации, наличие сегментированного генома (что облегчает рекомбинацию генов между различными вирусами гриппа) и случаев заноса зоонозных вирусов типа А [3].

Спектр эпидемических штаммов вирусов гриппа и их характеристика варьируют в зависимости от сезона года. В последнее время в Казахстане, как и во многих странах мира, наблюдается одновременная циркуляция вирусов гриппа подтипов A(H1N1), A(H3N2) и рода В [4-8].

Цель настоящего исследования состояла в изучении особенностей циркуляции вирусов гриппа в Аральском регионе Казахстана в эпидемические сезоны 2015-2017 гг.

Методы исследования. Сбор клинических образцов (назофарингиальные смывы, сыворотки крови) от больных осуществляли в поликлиниках и инфекционных больницах в эпидемические

периоды 2015-2017 гг. Актюбинской и Кызылординской областей. Пробы до вирусологических исследований хранили в жидком азоте.

Первичный скрининг носоглоточных смывов в полимеразной цепной реакции в режиме реального времени (РТ-ПЦР) осуществляли на амплификаторе RotorGen 6000 (CorbettResearch, Австралия) с применением наборов "РИБО – преп", "АмплиСенс® Influenzavirus A/B-FL" и "АмплиСенс ® Influenzavirus A-тип -FL" (производства ФБУН ЦНИИ эпидемиологии Роспотребнадзора, г. Москва) [9].

Изоляцию вирусов проводили в двух системах традиционными методами: на культуре клеток МДСК с добавлением ТРСК - трипсина (2 мкг/мл) и 9-11-дневных куриных эмбрионах (КЭ). Для индикации вирусов в реакции гемагглютинации (РГА) использовали 0,75% взвесь эритроцитов петуха и человека 0(1) группы крови.

Инфекционную активность изолятов определяли по общепринятому методу [10] и их титр выражали в lg $ЭИД_{50/0.2мл}$ и $lgТЦД_{50/0.2мл}$.

Идентификацию изолятов проводили в реакции торможения гемагглютинации (РТГА) и реакции ингибиции нейраминидазной активности (РИНА) с наборами поликлональных диагностических сывороток согласно рекомендации ВОЗ [11, 12].

Уровень специфических антител к вирусам гриппа в сыворотках крови определяли в РТГА и иммуноферментном анализе (ИФА). РТГА проводили согласно рекомендации ВОЗ с использованием как эталонных вирусов: A/California/04/09 (H1N1), A/Solomon Islands/03/06 (H1N1), A/USA/1976/31 (H1N1), A/Aichi/2/68 (H3N2), A/Panama/2007/99 (H3N2), B/Florida/04/06, так и коммерческих диагностикумов производства ФГБУ НИИ гриппа (г.Санкт-Петербург). Для ИФА использовали тест-системы производства ООО «ППДП» (г. Санкт-Петербург) к вирусам гриппа подтипов A(H1N1), A(H3N2) и типа B.

Результаты и обсуждение. Сбор материалов проводили в эпидемические сезоны 2015-2017 гг. в лечебных учреждениях Актюбинской и Кызылординской областей. Всего от больных людей было собрано 1978 смывов из верхних дыхательных путей и 127 сывороток крови.

Свыше 90% образцов собрано от пациентов с диагнозом острая респираторная вирусная инфекция. Наибольшее количество носоглоточных смывов (1291) получено от детей до 14 лет (65,27%).

В таблице 1 представлена характеристика собранного материала и результаты первичного скрининга носоглоточных смывов в РТ-ПЦР.

Таблица 1 – Характеристика и скрининг в РТ-ПЦР клинических образцов, собранных от людей в 2015-2017 гг.

Год	Место сбора	Количество носоглоточных смывов	Количество ПЦР-положительных проб				Количество сывороток крови
			к вирусу гриппа рода А	к вирусам подтипов:		к вирусу гриппа рода В	
				A/H1N1	A/H3N2		
2015	Актюбинская область	39	8	5	1	2	25
	Кызылординская область	254	29	3	26	1	23
Итого:		293	37	8	27	3	48
2016	Актюбинская область	17	4	3	2	0	22
	Кызылординская область	95	12	10	1	2	-
Итого:		112	16	13	3	2	22
2017	Актюбинская область	768	137	0	137	96	25
	Кызылординская область	805	25	0	25	80	32
Итого:		1573	162	0	162	176	57
Итого за 3 года		1978	215	21	192	181	127

Как видно из таблицы 1, при исследовании 293 проб, собранных в 2015 г., генетический материал вируса гриппа был обнаружен в 40 образцах (13,6 % случаев от общего числа проб). РНК вируса гриппа А выявлена в 37 пробах (12,6%), вируса гриппа В – в трех образцах (1,0%). Субти-

пирование позволило обнаружить РНК вируса гриппа A/H1N1 в восьми смывах (2,7% случаев), РНК вируса A/H3N2 в 27 пробах (9,2%).

Из 112 проб, полученных от больных людей в 2016 г., наличие генетического материала вируса гриппа в РТ-ПЦР выявлено в 18 образцах (16,1 % случаев от общего числа проб). РНК вируса гриппа А выявлена в 16 пробах (14,3%), вируса гриппа В – в двух образцах (1,8%). Субтипирование позволило обнаружить РНК вируса гриппа A/H1N1 в 13 смывах (11,6% случаев), в 3 смывах (2,7%) выявлена РНК вируса A/H3N2.

При исследовании 1573 биопроб, полученных в 2017 г., генетический материал вируса гриппа был обнаружен в 338 образцах (21,5 % от общего числа проб). РНК вируса гриппа А обнаружена в 162 биопробах (10,3%), вируса гриппа В – в 176 образцах (11,2%). Субтипирование ПЦР-положительных образцов на вирус гриппа типа А показало наличие генетического материала вируса гриппа A/H3N2 во всех 162 пробах, РНК вируса гриппа A/H1N1 выявить не удалось.

Таким образом, первичный скрининг носоглоточных смывов в РТ-ПЦР показал, что среди людей в Актюбинской и Кызылординской областях в 2015 – 2017 гг. социркулировали вирусы гриппа А и В. При этом вирус гриппа A/H3N2, преобладавший в 2015 г. и уступивший первенсто вирусу A/H1N1 в 2016 г., снова проявил себя в 2017 г.

В результате первичного заражения и последующих пассажей на КЭ и культурах клеток MDCK из ПЦР положительных проб выделено 13 гемагглютинирующих агентов с титрами на КЭ от 1:32 до 1:1024 и на культуре клеток MDCK – от 1:4 до 1:32.

Идентификацию изолятов 2015 – 2017 гг. выделения проводили в РТГА и РИНА. Результаты определения подтипа гемагглютинина изолятов приведены в таблице 2.

Таблица 2 – Идентификация подтипов гемагглютининов изолятов вирусов гриппа 2015-2017 гг. выделения в РТГА

Изолят	Титр антигемагглютининов иммунных сывороток					
	A/USA/1976/31 (H1N1)	A/Solomon Islands/03/06 (H1N1)	A/California/04/09 (H1N1)pdm	A/Aichi/2/68 (H3N2)	A/Panama/2007/99 (H3N2)	B/Florida/ 04/06
	1280*	**640**	**640**	**640**	**640**	**640**
Актобе/02/15	160	160	160	<20	<20	<20
Актобе/03/15	80	40	40	<20	<20	<20
Актобе/06/15	80	20	20	<20	<20	<20
Актобе/18/15	80	20	20	<20	<20	<20
Актобе/20/15	320	160	160	<20	<20	<20
Кызылорда /83/15	160	160	160	<20	<20	<20
Кызылорда/176/16	40	80	40	<20	<20	<20
Кызылорда/177/16	160	40	20	<20	<20	<20
Кызылорда/178/16	80	20	20	<20	<20	<20
Кызылорда/185/16	320	160	160	<20	<20	<20
Кызылорда/21/17	<20	<20	<20	<20	<20	80
Кызылорда/28/17	<20	<20	<20	<20	<20	160
Актобе/73/17	<20	<20	<20	<20	<20	80

*Представлены гомологичные титры референсных сывороток; гомологичный титр антител для референсной сыворотки к штамму A/USA/1976/31 (H1N1) составил 1:1280, для остальных – 1:640.

Из таблицы 2 видно, что гемагглютинирующая активность изолятов Актобе/02/15, Актобе/03/15, Актобе/06/15, Актобе/18/15, Актобе/20/15, Кызылорда /83/15, Кызылорда/176/16, Кызылорда/177/16, Кызылорда/178/16 и Кызылорда/185/16 от 1/32 до 1/4 гомологичных титров подавлялась иммунными сыворотками к вирусам A/USA/1976/31 (H1N1), A/Solomon Islands/03/06 и A/California/04/09 (H1N1)pdm. Это позволило отнести ГАА к вирусу гриппа А с подтипом гемагглютинина H1.

Гемагглютинирующая активность трех изолятов (Кызылорда/21/17, Кызылорда/28/17 и Актобе/73/17) от 1/8 до 1/4 гомологичного титра подавлялась иммунными сыворотками к вирусу гриппа B/Florida/04/06. С сыворотками к вирусу гриппа A/USA/1976/31 (H1N1), A/Solomon Islands/03/06 (H1N1), A/California/04/09 (H1N1)pdm и A/Aichi/2/68 (H3N2) получены отрицательные результаты, что позволило отнести изоляты 2017 г. к вирусу гриппа типа В.

Результаты идентификации подтипа второго поверхностного гликопротеида изолятов вируса гриппа А в РИНА представлены в таблице 3.

Таблица 3 – Идентификация подтипа нейраминидазы изолятов вируса гриппа 2015-2016 гг. выделения в РИНА

Изолят	Титр антител к подтипам нейраминидазы	
	N1	*N2*
А/Актобе/02/15	100	<20
А/Актобе/03/15	100	<20
А/Актобе/06/15	100	<20
А/Актобе/18/15	100	<20
А/Актобе/20/15	100	<20
А/Кызылорда /83/15	100	<20
А/Кызылорда/176/16	100	<20
А/Кызылорда/177/16	100	<20
А/Кызылорда/178/16	100	<20
А/Кызылорда/185/16	100	<20
Примечание. Приведены обратные величины титров антинейраминидазных антител.		

Из таблицы 3 видно, что нейраминидазная активность всех изолятов в титрах 1:100 подавлялась иммунной поликлональной сывороткой к вирусу A/H1N1.

Таким образом, по результатам РТГА и РИНА изоляты 2015-2016 гг. отнесены к вирусам гриппа А с антигенной формулой A/H1N1, а 2017 г. – к вирусу гриппа типа В.

Для изучения сероэпидемиологической ситуации по гриппу в Аральском регионе в 2015-2017 гг. в РТГА и ИФА исследовано 127 сывороток крови. Результаты РТГА приведены на рисунке 1.

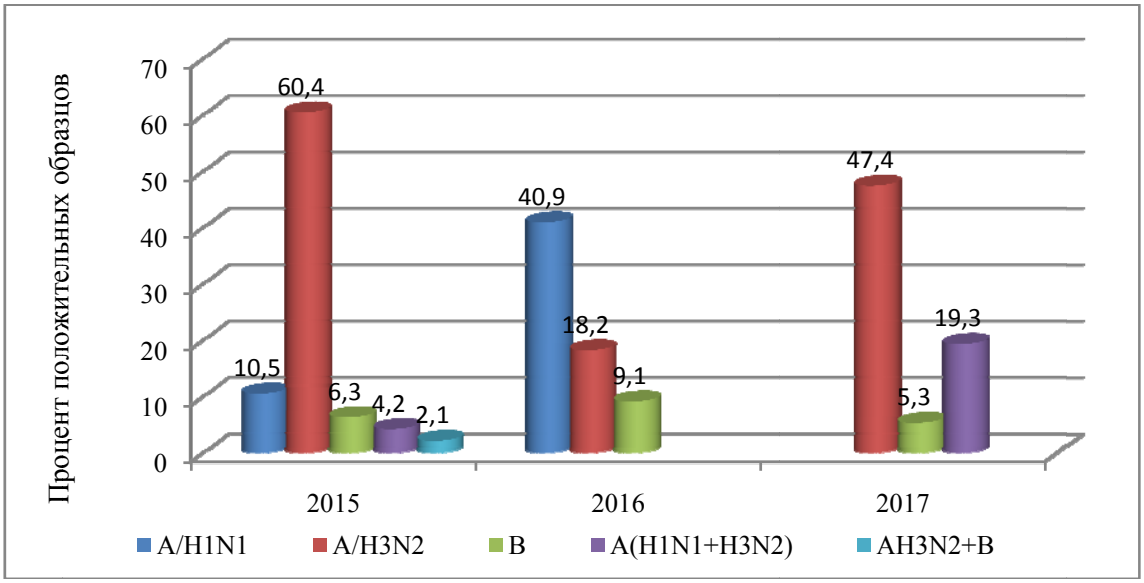

Рисунок 1 – Обнаружение специфических антител к вирусам гриппа в сыворотках крови в РТГА

Как видно из рисунка 1, в эпидемический сезон 2015 гг. в сыворотках крови людей в 60,4% (29 образцов) обнаружены антигемагглютинины к вирусу гриппа A/H3N2, в 10,5% случаев (пять проб) сыворотки оказались серопозитивными по отношению к вирусу гриппа A/H1N1. В 6,3% случаев (три образца) сыворотки были положительными по отношению к вирусу гриппа В, в 4,2% (две пробы) выявлены антигемагглютинины одновременно к вирусам гриппа A/H1N1 и A/H3N2, в 2,1% (одна проба) – к A/H3N2 и В. Титры антител составили 1:80 – 1:320.

В 2016 г. в 40,9% случаев (10 проб) в сыворотках крови людей обнаружены антигемагглютинины к вирусу гриппа A/H1N1, 18,2% случаев (четыре образца) оказались серопозитивными по отношению к вирусу гриппа A/H3N2. В 9,1% случаев (две сыворотки крови) были выявлены антигемагглютинины к вирусу гриппа В.

В 2017 г. в 47,4% случаев (27 проб) антигемагглютинины выявлены по отношению к вирусу серотипа A/H3N2, в 5,3% (три пробы) – к вирусу гриппа типа В, в 19,3% случаев (11 образцов) антитела обнаружены одновременно к вирусам гриппа A(H1N1 + H3N2). Титры антител составили 1:80 – 1:320.

На рисунке 2 представлены результаты серологического исследования 127 сывороток крови в ИФА.

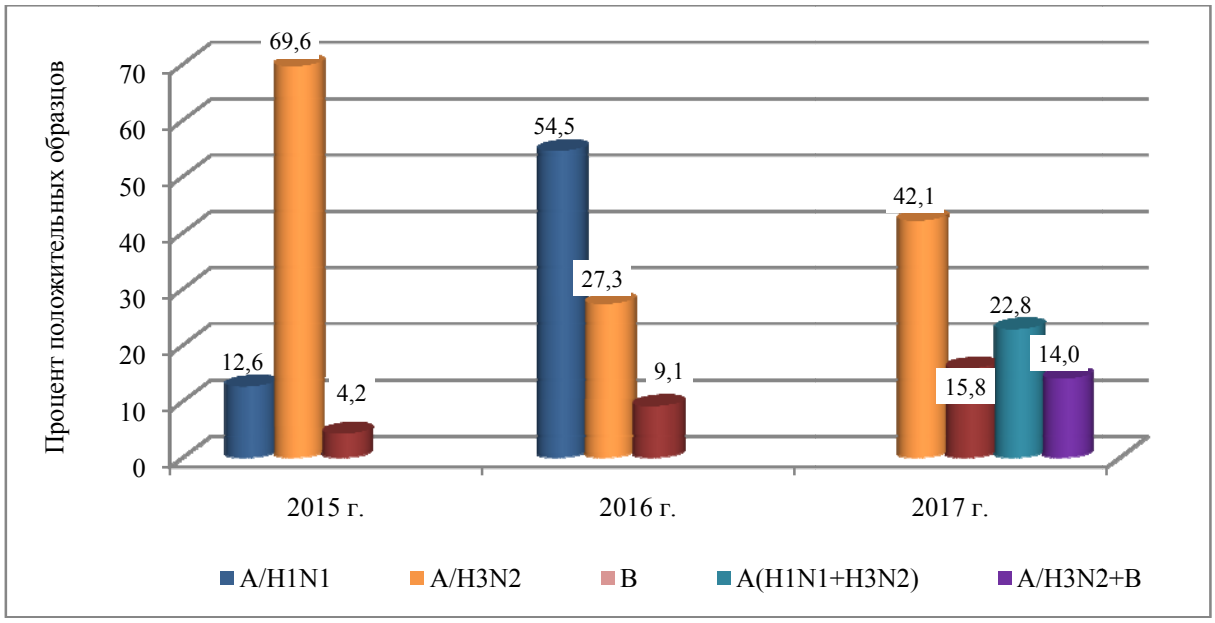

Рисунок 2 – Выявление антител к вирусам гриппа в сыворотках крови в ИФА

Как видно из рисунка 2, в эпидемический сезон 2015 г. антитела к вирусу гриппа A/H3N2 выявлены в 69,6% случаев (28 проб), к A/H1N1 – в 12,6% (шесть образцов) и к вирусу гриппа В – в 4,2% (две сыворотки).

В результате изучения 22 сывороток крови, полученных в 2016 г., антитела к вирусу гриппа A/H1N1 обнаружены в 54,5% случаев (12 образцов), к A/H3N2 – в 27,3% (шесть образцов) и к вирусу гриппа В – в 9,1% (две сыворотки крови).

В эпидемический сезон 2017 г. в подавляющем большинстве сывороток (42,1% - 24 образца) обнаружены антитела к вирусу гриппа A/H3N2, в 15,8% сывороток (девять образцов) – к вирусу гриппа В, в 22,8% случаев (тринадцать сывороток крови) выявлены антитела одновременно к вирусам A(H1N1 + H3N2), в 14,0% (восемь образцов) – к вирусам A/H3N2 и В.

Таким образом, результаты серологических исследований сывороток крови в ИФА и РТГА указывают на социркуляцию в Актюбинской и Кызылординской областях в эпидемические сезоны 2015-2017 гг. вирусов гриппа A/H1N1, A/H3N2, типа В и микст гриппозной инфекции. Отличительной особенностью эпидемического сезона 2017 г. является высокое содержание антител к вирусу гриппа A/H3N2 и В.

Согласно данным литературы, в последнее время наблюдается одновременная циркуляция штаммов – представителей различных эволюционных линий вирусов гриппа А и В [4, 13-16]. Причем антигенный состав вирусной популяции варьирует в зависимости от эпидемических сезонов [17-19]. Среди людей широко распространены подтипы вирусов гриппа А: H1N1 и H3N2. Вирус гриппа В протекает легче, чем тип А, дает небольшие вспышки и редкие мутации [20].

Уникальная антигенная вариабельность вирусов гриппа, позволяющая им преодолевать межвидовые барьеры, приводит к появлению вирусов с новыми биологическими свойствами, способных к широкому эпидемическому распространению [21]. В связи с этим крайне важными направлениями борьбы с гриппом являются надзор за распространением инфекции, своевременная диагностика возбудителя и профилактика заболевания.

Выводы. При первичном скрининге носоглоточных смывов и серологических исследований сывороток крови, собранных в эпидемический сезон 2015 -2017 гг. от больных людей в Актюбинской и Кызылординской областях, в РТ-ПЦР, РТГА и ИФА установлена социркуляция вирусов гриппа A/H3N2, A/H1N1 и В.

В результате вирусологических исследований из клинических образцов на КЭ выделено десять изолятов вирусов гриппа A/H1N1 и три - гриппа В, подтвердивших циркуляцию вирусов гриппа в данном регионе.

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2015-2017 ЖЖ. ТҰМАУ ІНДЕТІ АРАЛЫҒЫНДАҒЫ ҚАЗАҚСТАН РЕСПУБЛИКАСЫ АРАЛ МАҢАЙЫ ТҰРҒЫНДАР АРАСЫНДАҒЫ А ЖӘНЕ В ТҰМАУ ВИРУСТАРЫНЫҢ АЙНАЛЫМЫ

Аннотация. 2015-2017 жж. аралығында Ақтөбе және Қызылорда облыстарындағы инфекциялық емханаларымен поликлиникаларындағы сырқат адамдардан 2105 биосынамалар алынды. (1978 танау-мұрын жағындысы және 127 қан сарысуы).

Полимеразды тізбекті реакциясында адамдардан жиналған 1978 үлгіден А тұмау вирусының генетикалық материалы 10,86% жағдайында анықталды, В тұмау вирусы – 9,15%. А тұмау вирусын субтиптеу кезінде A/H1 тұмау вирусы – 9,76% сынамасында анықталса, A/H3 – 89,3% құрады.

Мұрын-танау жағындысын полимиразды тізбекті реакциясында скрининг жүргізу және қан сарысуын гемагглютинация тежеу рекциясымен иммуноферментті талдаудағы зерттеу нәтижелері, Ақтөбе және Қызылорда облыстарындағы адамдар арасында 2015-2017 жж. A/H1N1, A/H3N2 және В тұмау вирустары айналымда жүргендігін көрсетеді.

Адамдардан жиналған мұрын-танау жағындыларын вирусологиялық зерттеу нәтижесінде, тауық эмбриондарында 13 гемагглютининдеуші агент бөлініп алынды. Нейраминидаз белсенділігін тежеу реакциясы және гемагглютинация тежеу реакциясында 10 A/H1N1 тұмау вирусы, 3 В тұмау вирусы болып анықталды.

Вирусологиялық және серологиялық зерттеулердің нәтижелері Ақтөбе және Қызылорда облыстарындағы адамдар арасындағы тұмау індетін алдын-ала болжау және профилактикалық іс-шараларды жүргізу үшін, тұмау айналымын үздіксіз қадағалау қажеттілігін көрсетеді.

Түйін сөздер: айналым, тұмау вирусы, типасты, изолят, гемагглютинин, нейраминидаза, полимеразды тізбекті реакция, иммунноферметті талдау.

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STATE OF NATURAL FEEDING BASE OF FISH-BREEDING PONDS BY BREEDING OF FINGERLINGSOF THE PIKEPERCH

Abstract. Thepurpose of this work was a determination of the level and the dynamic ofdevelopment the phytoplankton and zooplanktonin experimental ponds which were used for breeding the fingerlingsof pikeperch in polyculture with common carp and plant-eating carps. An importance of studying the level and the dynamic of development the phytoplankton and zooplankton in ponds which were used for breeding the fingerlingsof pikeperch in polyculture with common carp and plant-eating carps is substantiated. The methods of studying the level and the dynamic ofdevelopment the phytoplankton and zooplanktonin experimental ponds are presented. The level of phytoplankton and zooplanktonin experimental ponds in which was hold breeding the one-years of pikeperch from the fingerlings,is shown. The composition of species, the level of development of phytoplankton and zooplanktonin experimental ponds in different years of researchesis shown. The dynamics of the level of development of phytoplankton and zooplanktonin some months of the determined year of holding the researchesis shown. The fact that holding of measures according to the stimulation of development the natural feeding base which are the using the fertilizers etc. is influencing for increasing the biomass of organisms of zooplankton especially in the end of fish-breeding season, is shown. The conclusions in which presented the dynamic of development of phytoplankton and zooplankton of experimental ponds in different months of the year are given. The importance of hydrobiological researches by breeding the one-years of pikeperch in fish-breeding ponds is shown. The recommendations according to the results of researches the dynamic and biomass of phytoplankton and zooplankton are given. The period of fish-breeding season in which holding the works according to the maintenance of biomass of phytoplankton and zooplankton in fish-breeding ponds which are using for the breeding of one-years of pikeperch is recommended.

Keywords: phytoplankton, zooplankton, dynamicof development, fish breeding in ponds, one-years, pikeperch.

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СОСТОЯНИЕ ЕСТЕСТВЕННОЙ КОРМОВОЙ БАЗЫ ПРУДОВ ПРИ ВЫРАЩИВАНИИ СЕГОЛЕТОК СУДАКА

Аннотация. Целью работы было определение уровня и динамики развития фитопланктона и зоопланктона в экспериментальных прудах, занятых под выращивание сеголеток судака в поликультуре с карпом и растительноядными рыбами. Обоснована важность изучения уровня развития фито- и зоопланктона в прудах, занятых под выращивание сеголеток судака в поликультуре с карпом и растительноядными рыбами. Представлены методики изучения уровня и динамики развития фитопланктона и зоопланктона в экспериментальных прудах. Показан уровень развития фито- и зоопланктона в экспериментальных прудах, в которых проводилось выращивание сеголеток судака от подрощенной молоди. Показаны видовой состав, уровень развития организмов фито- и зоопланктона экспериментальных прудов в разные года проведения исследований. Показана динамика уровня развития фито- и зоопланктона в отдельные месяцы определенного года проведения исследований. Показано, что проведение мероприятий по стимуляции развития есте-

ственной кормовой базы (внесение удобрений и др.) оказывает непосредственное влияние на увеличение биомассы организмов зоопланктона, особенно к концу сезона эксплуатации рыбоводных прудов. Даны выводы, в которых представлена динамика развития фито- и зоопланктона экспериментальных прудов по месяцам года, показано значение гидробиологических исследований при выращивании сеголеток судака в прудах. По результатам исследований динамики и биомассы фито- и зоопланктона даны рекомендации, в какой период рыбоводного сезона наиболее целесообразно проводить работы по поддержанию биомассы фито- и зоопланктона в рыбоводных прудах, занятых под выращивание сеголеток судака.

Ключевые слова: фитопланктон, зоопланктон, динамика развития, прудовое рыбоводство, сеголетки, судак.

Введение. В настоящее время, при возрождении в Казахстане аквакультуры как отрасли, одним из путей развития рыбоводных предприятий, в частности, прудовых хозяйств, является освоение производства рыбопосадочного материала новых объектов аквакультуры с целью зарыбления естественных водоемов для воспроизводства промысловых популяций, а также для выращивания товарной рыбной продукции.

Одним из технологических параметров в прудовом рыбоводстве является биомасса фито-, зоопланктона и макрозообентоса при выращивании рыбы на протяжении рыбоводного сезона, на основании которой можно получить косвенную информацию о кормности пруда, что позволяет оптимально планировать мероприятия по ее повышению, что в конечном счете дает повышение рыбопродуктивности прудов, как правило, до определенного предела, без дополнительных затрат искусственных кормов. Благодаря этому увеличивается экономическая эффективность прудового рыбоводства.

Одним из новых объектов рыбоводства Казахстана является судак. Высокие вкусовые качества этой рыбы позволяют осуществлять экспорт значительной части уловов казахстанского судака в страны Европы. При этом особую актуальность приобретает воспроизводство запасов судака в рыбохозяйственных водоемах страны. Исследования ТОО «Казахский научно-исследовательский институт рыбного хозяйства» показали возможность выращивания сеголеток судака в прудах карповых рыбоводных хозяйств в поликультуре с двухлетками карпа и растительноядных рыб.

Состояние естественной кормовой базы прудов при выращивании судака как объекта аквакультуры представляет большой научный и практический интерес.

Материал и методика

По материалам, полученным рыбоводами Венгрии, наибольшее значение для молоди судака имеют фитопланктон и зоопланктон. Развитие фитопланктона способствует повышению биомассы мельчайшего зоопланктона (Rotatoria), а затем – более крупных форм кормовых планктонных ракообразных (Cladocera, Copepoda) [1].

Материалом для исследований служили видовой состав, численность и биомасса фитопланктона и зоопланктона экспериментальных прудов, задействованных под выращивание сеголеток судака в поликультуре с двухлетками карпа, белого амура и белого толстолобика.

Исследование состояния фитопланктона и зоопланктона экспериментальных прудов проводили по стандартным методикам, принятым при гидробиологических исследованиях [2-5].

Состояние естественной кормовой базы экспериментальных прудов сравнивали с ростом сеголеток судака и уровнем рыбопродуктивности по данному объекту аквакультуры [1, 6-20].

Результаты и их обсуждение

Фитопланктон. По результатам обработки проб, отобранных в экспериментальных прудах в весенне-летний период 2013-2014 гг., выявлено 52 вида водорослей, относящихся к 6 отделам, среди которых: синезелёных - 10, диатомовых - 23, зелёных - 12, пирофитовых - 3, эвгленовых - 3, золотистых - 1. Доминирующий комплекс фитопланктона составляли следующие виды водорослей: N.gregaria, Achnanthes sp., C. vulgaris, P. achromaticum, C. meneghiniana, E. cordata, P. achromaticum, Trachelomonas sp. Наименьшее количество видов водорослей встречается в прудах в мае. В летний период, т.е. в июне-июле месяцах в прудах фитопланктон развивается более интенсивно.

Динамика показателей биомассы фитопланктона в экспериментальных прудах в сезонах 2013-2014 гг. представлена в таблице 1.

Таблица 1 – Динамика количественного развития (биомассы, г/м^3) фитопланктона в экспериментальных прудах в 2013-2014 гг.

Отделы водорослей	2013				2014			
	май	июнь	июль	август	май	июнь	июль	август
Зеленые	–	0,040	0,250	0,020	0,085	0,015	0,020	0,030
Синезеленые	0,020	0,155	0,225	0,001	0,008	0,045	0,039	0,055
Диатомовые	0,065	0,955	2,00	0,855	0,050	0,900	0,645	0,400
Эвгленовые	–	0,150	0,105	0,075	0,040	0,950	0,040	0,200
Пирофитовые	0,015	0,215	0,475	0,100	0,200	0,050	0,070	0,400
Золотистые	–	–	–	–	0,010	–	–	–
В среднем	0,100	1,515	3,055	1,051	0,393	1,960	0,814	1,085

В весенний период фитопланктон в двух сезонах был развит слабо, по величине биомассы фитопланктона в мае пруды отнесены к водоёмам низкого класса кормности β-олиготрофного типа.

По величине биомассы фитопланктона в июне пруды можно считать водоёмами умеренного класса кормности α-мезотрофного типа.

В июле 2013 г. пруды по показателю биомассы водорослей соответствовали водоёму среднего класса кормности β-мезотрофного типа, в июле 2014 г. пруды - водоёму умеренного класса кормности β-мезотрофного типа. В августе пруды по величине биомассы фитопланктона можно было считать водоёмами умеренного класса кормности α-мезотрофного типа [5].

По «шкале трофности» С. П. Китаева пруды в июне по величине биомассы фитопланктона соответствовали среднему классу кормности, β-мезотрофного типа. В июле-августе класс кормности пруда стал соответствовать умеренному, а тип водоёма – α- мезотрофному [5].

Основу биомассы фитопланктона в июне создавали эвгленовые водоросли (54,8 %), в июле – диатомовые (56,9 %),а в августе – пирофитовые (55,5 %). Таксономический список водорослей, отобранных в экспериментальных прудах в 2015 г. насчитывал 62 вида водорослей, относящихся к 5 отделам. Среди них: синезелёных - 14, диатомовых - 22, зелёных - 20, пирофитовых - 4, эвгленовых - 2.

Доминирующий комплекс фитопланктона представлен следующими видами водорослей: N. Gregaria, C. meneghiniana, A. ovalis, C. vulgaris, C. undulatum, Trachelomona ssp., E. cordata. Наименьшее количество видов водорослей было зарегистрировано в мае-июне и варьировало от 4 до 9 таксонов. Таксономический состав водорослей варьировал от 13 до 21 таксонов. В весенний период фитопланктон был развит слабо, показатели биомассы были невысокими и варьировали от 0,265 до 0,691 г/м^3.

Состав доминирующих видов различался в течение летнего периода. Так, в июне в водоёме доминировали пирофитовые (56 %), в июле – диатомовые (34,4 %), а в августе – синезелёные водоросли (42 %).

В июне – июле 2015 г. показатели биомассы фитопланктона (0,365–2,665 г/м^3) соответствовали умеренному классу кормности, α- мезотрофному типу. В июне основу биомассы создавали пирофитовые водоросли (71,4 %), а в июле – диатомовые (35,7 %). В августе уровень кормности водоёмов повысился до среднего класса, а тип водоёма до β-мезотрофного (биомасса – 0,360–3,015 г/м^3). Основу биомассы фитопланктона в это время создавали диатомовые водоросли (62 %).

Зоопланктон. По результатам гидробиологической съемки в период апрель-август 2013 г. зоопланктон экспериментальных прудов представлен 55 таксонами из трех основных групп, где 26 таксонов – коловратки, 16 ветвистоусые и 13 веслоногие рачки. Помимо зоопланктонных организмов в пробах встречено большое количество факультативных зоопланктеров – остракоды, личинки насекомых, черви, гидры.

Наибольшее таксономическое разнообразие встречено в пруду №3 – 43 таксона, из которых 21 коловратка, 12 ветвистоусых и 10 веслоногих рачка. В пруду №4 выявлено соответственно 31-16-9-6 таксонов. Основной фон зоопланктонного сообщества в обоих прудах составили (33-45 % встречаемости за весенне-летний период) *S. pectinata, A. sieboldi, L. ungulate, E. d. dilatata, C. laticaudata, S. mucronata, Ch. sphaericus и M.leuckarti.* В пруду №3 к ним добавились *E. pyriformis, Br. q. melheni, D. macrophthalma, P. Trigonellus,* в пруду №4 – *C. reticulate, N. incongruens*.

В пруду №4 основу численности и биомассы составили ветвистоусые ракообразные – 75,4 % по числу экземпляров и 84,3 % по биомассе. Продукционные показатели на м^3 в пруду начали расти с июня месяца и до середины августа не опускались ниже 65,0 тыс. экземпляров и 1,5 г/м^3, достигнув своего пика в начале июля – 263,7 тыс. экземпляров и более 9,0 г/м^3, за счет массового развития ветвистоусых рачков рода Ceriodaphnia, доля которых в общих показателях составила от 30 до 94 %.

В пруду №3 основу численности составили веслоногие ракообразные – 41,6 %, биомассу в водоеме сформировали ветвистоусые рачки – 48,2 %. За сезон наибольшие продукционные показатели выявлены в мае месяце, за счет массового развития крупных представителей ветвистоусых рачков D. galeata, S. vetulus, S. mucronata, и в переходном периоде июль-август, за счет развития крупных хищных коловраток рода *Asplanchna*, ветвистоусого рачка C. laticaudata и младших возрастных стадий веслоногих рачков (науплиусы и копеподы).

Гидробиологический анализ естественной кормовой базы экспериментальных прудов в сезонах 2012–2014 гг. показал, что зоопланктон представлен 55 таксонами из трех основных групп, где 26 таксонов – коловратки, 16 ветвистоусые и 13 веслоногие рачки. Помимо зоопланктонных организмов, в пробах встречено большое количество факультативных зоопланктеров – остракоды, личинки насекомых, черви, гидры.

Определяющая роль в планктоне всех прудов в течение вегетационных сезонов принадлежала ветвистоусым рачкам. Отмечалось устойчивое доминирование их в общей массе зоопланктона в экспериментальных прудах.

Анализируя динамику уровня развития зоопланктона в течение сезона в 2012-2014 гг. в экспериментальных прудах, можно отметить, что после залития опытных прудов количественные показатели численности и биомассы зоопланктеров в них были идентичными низкими и находились в пределах 19,04–39,76 тыс. экз./м^3 и 0,355–0,827 г/м^3 соответственно.

Динамика количественных показателей зоопланктона в экспериментальных прудах в 2012-2014 г. представлена в таблице 2.

Таблица 2 – Динамика численности и биомассы зоопланктона в экспериментальных прудах в 2012-2014 г.

Дата	2012		2013		2014	
	тыс. экз/м^3	г/м^3	тыс. экз/м^3	г/м^3	тыс. экз/м^3	г/м^3
14.04.	25,7	0,827	39,760	0,640	19,04	0,355
28.04.	89,5	1,738	10,90	0,126	29,860	1,485
15.05.	129,1	3,817	246,60	4,441	136,70	5,460
31.05.	101,0	1,864	65,970	1,763	63,50	2,189
14.06.	112,1	4,367	93,450	2,579	55,040	2,009
29.06.	129,1	3,817	87,920	3,071	53,0	1,962
15.07.	155,5	2,163	84,511	2,823	31,68	1,660
30.07.	165,0	2,792	98,176	2,740	10,87	0,10
15.08.	76,5	1,349	17,10	0,382	8,80	0,069

Для повышения уровня естественной кормовой базы в прудах были проведены интенсификационные мероприятия, которые стимулировали развитие гидробионтов. Были внесены: органические удобрения (навоз крупного рогатого скота) из расчета 2 т/га; минеральные удобрения (аммиачная селитра из расчета 20 кг/га; суперфосфат – 10 кг/га); снопы подвяленной высшей водной растительности (тростник, рогоз); маточная культура дафнии (1 л/га); кормовые дрожжи (1 кг/га).

Результаты, полученные после стимуляции, указывают на общую тенденцию роста количественных показателей, которые достигают своего максимума в 2012-2014 гг. в I декаде мая (129,1-246,6 тыс.экз/м^3 и 3,817-5,46 г/м^3 соответственно). Данные показатели характеризуют экспериментальные пруды в этот период, как высококормные [5].

По показателям количественного развития зоопланктона в сезонах 2012 и 2013 гг. в целом пруды были высококормными. В сезоне 2014 гг. динамика развития зоопланктеров характеризует пруды как среднекормные [5]. Вероятно снижение количественных показателей планктона связано с увеличением на пруды нагрузки по карпу. В 2014 году плотность посадки годовиков карпа в поликультуре с судаком составила 1000 шт/га. Как известно, зоопланктон у годовиков карпа составляет основу пищевого рациона в первую половину рыбоводного сезона.

К концу сезона кормность всех прудов в сезонах 2012-2014 гг. снижается. По классификации кормности все пруды в этот период соответствовали низкокормным [5].

По результатам гидробиологических исследований в весеннее-летний период 2015 года зоопланктон прудов Чиликского прудхоза представлен 66 таксонами из трех основных групп, где 32 таксона – коловратки, 16 ветвистоусые и 18 веслоногие рачки. Основной фон зоопланктонного сообщества в обоих прудах составили *A. girodi, A. sieboldi, A. brightwelli, L. bulabula, Br. c. Amphiceros, D. macrophthalma, C. reticulata, C. quadrangula, M. brachiata, S. mucronata, D. crassa, Ch. sphaericus, A. americanus и M. leuckarti*(57-100 % встречаемости за весенне-летний период).

Если рассматривать по месяцам, то наибольшее число видов по прудам выявлено июне-июле месяцах 46-44 таксона соответственно, наименьшее в августе – 22 вида. Количественное развитие зоопланктона в прудах представлено в таблице 3.

Основу численности и биомассы в пруду №3 в среднем составили ветвистоусые ракообразные – 74,5 и 87,0 % соответственно. На протяжении всего вегетационного периода они преобладали в сообществе, однако в начале июня доминантной группой стали коловратки, где основу заложили хищные коловратки рода *Asplanchna* (42,7 % по численности и 72,9 % по биомассе). За сезон наибольшие продукционные показатели выявлены в период – середина июня-середина июля, с пиком в середине июля месяца (14,929-12,369-21,369 г/м^3), когда биопродуктивность пруда достигала высокого-очень высокого класса кормности с евтрофным-гипертрофным типом за счет массового развития ветвистоусых рачков рода *Ceriodaphnia* (81,3-92,5-95 % от общей биомассы). В мае в пробах отмечено большое количество факультативных планктонных организмов, где наибольшее значение имели остракоды, доля которых в общей биомассе составила около 26,0 %.

Основу численности и биомассы в пруду №4 составили также ветвистоусые ракообразные – 92,1 и 96,8 % соответственно. Наибольшие показатели биомассы выявлены в июне месяце – 22,121-22,627 г/м^3 и в середине июля (32,528 г/^{3}), где основу составили ветвистоусые рачки рода *Ceriodaphnia* (97,4-98,0-97,1 % от общей биомассы).

Основу численности и биомассы в пруду №1 составили ветвистоусые ракообразные – 74,5 и 87,0 % соответственно. За вегетационный период максимальные продукционные показатели выявлены в период – конец июня-конец июля, с пиком в середине июля месяца (3,156-5,830-3,220 г/м^3), за счет массового развития ветвистоусых рачков рода *Ceriodaphnia* и *Moina* (46,4-53,9-51,8 % от общей биомассы).

Основу количественных показателей в пруду №5 за период исследования составляли ветвистоусые рачки – 61,1 % по числу экземпляров и 66,8 % по биомассе. Биомасса зоопланктона за сезон варьировала от 3,923 г/м^3 в конце июне до 8,089 г/м^3 в конце июля, что соответствует повышенному и высокому классу кормности с евтрофным типом водоема. Среди видов доминировали рачки рода *Ceriodaphnia* – 42,8 %, коловратки рода *Asplanchna* – 16,2 %, *M.brachiata* – 10,9 %.

За период исследования во всех прудах доминировали ветвистоусые ракообразные, среди них рачки рода *Ceriodaphnia,* доля которых в общей массе доходила до 98 %.

Из исследованных водоемов наиболее продуктивными по зоопланктону оказались пруды №3 и №4, где показатели варьировали от среднекормного класса мезотрофного типа до очень высококормного класса гипертрофного типа. Продуктивность зоопланктона прудов №1 и №5 была несколько ниже, показатели колебались от низкокормного класса олиготрофного типа до высококормного евтрофного типа [5].

Таблица 3 – Количественное развитие зоопланктона в экспериментальных прудах в сезоне 2015 г.,
ч – численность, тыс. экз./м^3, б – биомасса,г/м^3

Дата отбора проб	Коловратки		Ветвистоусые		Веслоногие		Итого	
	ч	б	ч	б	ч	б	ч	б
Пруд №3								
20.05	11,90	0,116	76,0	5,027	30,0	0,663	117,90	5,806
05.06	235,90	5,337	54,80	1,268	32,20	0,638	322,90	7,243
20.06	10,30	0,128	430,0	14,401	59,50	0,413	499,80	14,942
05.07	15,0	0,082	331,0	11,915	44,30	0,249	390,30	12,246
20.07	20,60	0,091	500,0	20,757	38,0	0,511	558,60	21,369
05.08	1,20	0,021	96,0	3,407	10,30	0,263	107,50	3,691
Средний показатель	49,150	0,963	247,967	9,463	35,717	0,456	332,834	10,882
Пруд №4								
20.05	1,80	0,002	95,0	7,416	21,20	0,105	118,0	7,523
05.06	24,0	0,045	875,20	21,899	33,60	0,177	932,80	22,121
20.06	8,0	0,027	644,0	22,290	48,30	0,310	700,30	22,627
05.07	6,0	0,126	128,90	4,959	9,30	0,053	144,20	5,138
20.07	3,0	0,007	879,80	32,133	32,60	0,388	915,40	32,528
05.08	20,0	0,10	195,0	5,672	33,80	1,825	248,80	7,597
Среднее показатель	10,467	0,051	469,650	15,728	29,80	0,476	509,917	16,255
Пруд №1								
12.06	15,20	0,070	23,50	0,711	6,20	0,030	44,90	0,811
27.06	22,60	1,052	39,80	1,545	25,20	0,559	87,60	3,156
12.07	59,0	1,619	80,40	3,301	45,20	0,910	184,60	5,830
27.07	23,30	0,388	56,30	1,856	22,0	0,976	101,60	3,220
12.08	9,20	0,216	33,0	1,257	32,60	0,197	74,80	1,670
Средний показатель	25,860	0,669	46,60	1,734	26,240	0,534	98,70	2,937
Пруд №5								
12.06	2,40	0,005	213,60	5,306	30,60	0,350	246,60	5,661
27.06	42,60	1,514	48,40	1,80	14,40	0,609	105,40	3,923
12.07	34,0	0,649	69,60	2,548	39,90	1,190	143,50	4,387
27.07	64,0	2,325	122,0	4,769	52,0	0,995	238,0	8,089
12.08	12,0	0,245	87,0	3,726	52,0	1,139	151,0	5,110
Среднее	31,0	0,947	108,12	3,630	37,780	0,857	176,90	5,434

На необходимость повышения уровня развития зоопланктона в прудах, где производится выращивание сеголеток судака, указывают также российские и белорусские ученые [6-9].

Как правило, повышение уровня развития зоопланктона в первую половину рыбоводного сезона является залогом повышения рыбопродуктивности по сеголеткам судака. Во вторую половину рыбоводного сезона зоопланктон играет роль фонового (второстепенного) объекта питания сеголеток судака. Основным объектом питания в данный период времени становится молодь сорных видов рыб [10-13].

Как видно из представленных данных, наибольший рост биомассы фитопланктона наблюдается в период «май - июнь», наибольший уровень развития зоопланктона приходится на июль-месяц, затем, в августе-месяце, следует спад биомассы фитопланктона.

Выводы.

1) В целом по результатам гидробиологических исследований естественной кормовой база экспериментальных прудов было установлено, что уровень развития биомассы фитопланктона и зоопланктона был оптимальным для выращивания сеголеток судака.

2) В начале рыбоводного сезона, сразу после залития прудов, уровень развития фито- и зоопланктона, как правило, низкий. Учитывая раннее зарыбление прудов подрощенной молодью судака, которое в условиях прудовых хозяйств юга Казахстана приходится на начало мая, необходимо проводить более раннее залитие выростных прудов и осуществлять комплекс мероприятий по повышению уровня развития фито- и зоопланктона.

3) Поддерживать развитие фито- и зоопланктона необходимо в первую половину рыбоводного сезона. В дальнейшем уровень развития естественной кормовой базы прудов является достаточно высоким.

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ОСЫ ЖАДЫҚ КӨКСЕРКЕ БАЛЫҒЫН ӨСІРУДЕГІ ТОҒАНДАРДЫҢ ҚОРЕКТІК БАЗАСЫНЫҢ ТАБИҒИ КҮЙІ

Аннотация. Жұмыстың мақсаты тұқы мен шөппен қоректенетін балықтармен поликультура жағдайында осы жаздық көксерке балықтары өсірілген тәжірибелік тоғандардағы фитопланктон мен зоопланктонның даму динамикасы мен деңгейін анықтау болды. Мақалада осы жаздық көксерке балықтарын тұқы мен шөппен қоректенетін балықтармен бірге өсірген кездегі тоғандардың фито- және зоопланктон даму деңгейін зерттеу маңыдылығы көрсетілген. Тәжірибелік тоғандардағы фитопланктон мен зоопланктон даму динамикасы мен деңгейін зерттеудің әдістемелері келтірілген. Фито- және зоопланктон даму динамикасы зерттелген тәжірибелік тоғандарда осы жадық көксерке балықтары өскелең шабақ кезеңінен бастап өсірілген. Тәжірибелік тоғандардағы бірнеше жылдық зерттеу жұмыстарын жүргізу барысындағы фито- және зоопланктон ағзаларының даму деңгейі мен түрлік құрамы көрсетілген. Белгілі бір жылдарда зерттеу жұмыстарын жүргізген кезеңдердегі, жекелеген айлардағы фито- және зоопланктон даму деңгейінің динамикасы көрсетілген. Табиғи қоректік базаны қолдан арттыру үшін жасалатын іш шаралар (тыңайтқыштарды салу және т.б.) тікелей зоопланктон биомассасын арттыруға оң әсер ететіндіктері анықталған, әсіресе балық өсіретін тоғандарды пайдалану маусымының соңына қарай. Қортындыда тәжірибелік тоғандардағы фито- және зоопланктон даму динамикасы айма-ай көрсетілген, тоғандарда осы жаздық көксерке балығын өсірудегі гидробиологиялық зерттеу жұмыстарының маңыздылығы баяндалған. Фито- және зоопланктон динамикасы мен биомассасын зерттеу нәтижелері бойынша балықты қолдан өсіру маусымында осы жаздық көксерке балығын өсіру барысында фито- және зоопланктон биомассасын біркелкі әрі тұрақты етіп ұстап отыру үшін қажетті ұсыныстар берілген.

Түйін сөздер: фитопланктон, зоопланктон, даму динамикасы, тоған балық шаруашылығы, осы жадық балықтар, көксерке.

МАЗМҰНЫ

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(ағылшын тілінде)

Медицина
(орыс тілінде)

Биология
(ағылшын тілінде)

Биология
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