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Research Article

Method for Vitreous Body Microsurgical Anatomy Studying

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Abstract

Purpose: The vitreous body (VB), due to the complexity of its structure, remains one of the least studied anatomical structures to this day. In literature there are attempts to describe the anatomy of the vitreous body, since the II century. The most relevant works are the studies of J. Worst., *et al*. in 1973, in which the authors proposed new methods of CT preparation with the introduction of dyes. Despite many years of research on the structure and functions of the vitreous body and the presence of a large number of works, and there are no methods and protocols for macromicroscopic examination of the vitreous body to develop a method and propose a protocol for macromicroscopic examination of the vitreous body (VB), allowing to obtain new data on VB topographic anatomy.

The purpose of the study is to develop the algorithm of examination of the vitreous body microsurgical anatomy that would let to assess its topographic specifics.

Materials and Methods: The proposed method of macromicroscopic examination was used to study the VB topographic anatomy of 38 cadaver eyeballs. In order to color transparent structures of the vitreous, poorly soluble metallic salts barium sulfate (Vitreocontrast) was used. Macroscopic examination was performed using a TopconOMS-800 operating microscope with a magnification of x8 to x21, microscopic changes were evaluated by light microscopy at x50, x100, x200, x400 x630 multiple magnification with Leica DM LB2 microscope followed by photographic recording. The algorithm for macroscopic examination performing.

Results and Discussion: The result of macroscopic preparation was the compilation of individual anatomical and topographic maps of VB patients. A distinctive feature of the developed method is the ability to dissect any VB structure and to isolate each cortical layer with the possibility of studying its anatomical and topographic features and relationships with underlying tissues (internal limiting membrane, ciliary body, lens capsule). In addition, the method allows to maintain the shape and integrity of the specimens after passing through all stages of histological processing. In order to fixate VB samples, we used a method with fixing VB structures on a special adhesive-metric tablet, and placing them in a biopsy bag placed in a biopsy cassette. After that, filled in formalin, the specimens were delivered to the laboratory, where all the stages of standard processing took place.

Conclusion: The developed technique of macromicroscopic examination of the vitreous allows to create an individual map of the VB topographic anatomy. After collecting of sufficient material and its statistical processing, it is possible to provide maps of the VB topographic anatomy in normal, age-related and pathological conditions.

Keywords: Macromicroscopic Examination; Vitreous Body; Vitreoretinal Interface; "Vitreocontrast"; Vitreolenticular Interface

Introduction

Until now, the vitreous body is one of the least studied anatomical structures. The first attempts to study the anatomy of the vitreous body were made on the turn of the II-nd century. Galen described the vitreous body based on the works by the great anatomists of Alexandria such as Rufus of Ephesus [1]. According to S.W. Duke-Elder the first attempts to make the theory of the vitreous body and to describe its structure date back to the mid of the 18-th century [2]. The author thought that the vitreous body consists of densely packed collagen plates forming special structural components – tracts: the retrolental tract, the anterior ciliary tract, the posterior ciliary tract, and preretinal tract [5]. In the 18-19-th centuries there were four different theories of the vitreous body structure – the alveolar one (P.Demours), the lamellar one (J.G.Zinn), the radial sector one (A.Hannover), and the fibrilar one (William Bowman.) 1741 г. Pierre Demours moved the alveolar theory of the vitreous body structure assuming that between the fabrlious vitreous structures there are alveoli filled with liquid. In 1780, Johann Gottfried Zinn supposed that the vitreous body was a complicated arranged structure, the lamellae of which were concentrically arranged and reminded the structure of an onion. The data received by Von Pappenheim and Brucke after the preparation and the histological investigation of the VB confirmed J.G.Zinn lamellar theory of VB structure. In 1845, Adolph Hannover formulated the third theory – radial sector theory. Studying VB sections in the equator area, he described numerous sectors radially oriented around the central zone where Cloquet's canal is located. The structure of the VB reminded him of a "sliced orange" [6,7]. In 1848, William Bowman using microscopy discovered fibrils having a wave-like course in the central part of the VB and forming knots (intersection points of threads visible in microscopy) and introduced the concept of "fibrilar" theory for the first time [8]. In 1932, Redslob used a slit lamp for the vitreous body examination. The application of microscopy in the dark field gave a more complete picture of its structure.

However, despite many years of research on the structure and functions of the vitreous body and a large number of studies of the eye, the vitreous body still remains the least studied structure of the eyeball due to its complex microstructure. Professor J.Worst., *et al*. practically opened a new era in the study of the vitreous body. He was the first to introduce various vital dyes into the vitreous body to examine its structure. These authors developed methods of VB dissection on isolated eyes by the type of "flower", "window" and "hammock". These methods consisted in the separation of the vitreous body followed by staining of its structures. It was found that the vitreous body, despite its gel-like properties, retained its shape even when completely extracted from the eye. This gave grounds to suggest the existence of its own outer tunic or compacted marginal zone, the authors also described three rows of cisterns (ring of equatorial, retrociliary and petaliform cisterns); canals (lentico-macular, optico-ciliary), premacular bursa and other structural elements of the VB [9,10]. It should be noted that the methods of VB dissection proposed by the authors had a number of disadvantages, since they involved the removal of the cornea, iris, lens, which violated the integrity of VB structures. The petals of the sclera, choroid and retina complicated visualization and assessment of anatomico-topographic features of the VB structure. During VB dissection, it was impossible to stain, separate and isolate VB structures separately, since the Magic color dyes that were used had poor adhesion to VB structural elements, and they did not stay in the cavities of canals and cisterns.

Until now, the method of contrast staining and filling cavities with different dyes was practically the only one that did not only enabled to determine structures but also to obtain the picture of anatomico-topographic peculiarities of their location.

However, the introduction of known dyes (Magic color, ink, triamcinalone acetonide or water-soluble dyes) did not bring researchers closer to the practical possibility of making anatomical sections of the vitreous body and micro preparations for performing light microscopy.

Microsurgical anatomy is a branch of clinical anatomy that studies the structure and topography of small anatomical structures of organs and body regions in norm and pathology as applied to the needs of microsurgery. The main feature of microsurgical anatomy is the study of anatomical structures in the macromicrosurgical field of vision, i.e. in the range of magnification of stereoscopic microscope, surgical magnifiers and surgical microscope. Microsurgical anatomy combines two principles - the use of macromicroscopic fields of vision corresponding to the range of the operating microscope and the topographic principle of studying the structure and location of anatomical structures. The methodological basis of microsurgical anatomy study is a complex of

techniques, in which the leading place belongs to macromicroscopic dissection and histotopographic method. Macromicroscopic dissection in a classical variant is a stereomorphological method of preparation developed by the academician V.P. Vorobyov. Such dissection was carried out by microsurgical instruments with the chosen direction of illumination source and allowed receiving materials on macromicroscopic structure of blood supply of peripheral nerve plexuses. Currently, macromicroscopic dissection is performed using stereomicroscopic microscopes, which have a much larger field of vision, a wide range of magnifications from 3.6 to 98.0, various options of object illumination, convenient opportunities both for dissection and study of finished anatomical specimens and histotopographic slides in incident and transmitted light. The second method of microsurgical anatomy research is histotopographic. The histotopographic method uses a series of histotopograms of different but precisely fixed levels. Their planes are spatially precisely oriented. They are studied as a whole, which allows reconstructing topographic relations in extent and in space. A histotopogram is understood as a stained histological section of an atomic entity or organ. To prepare histographic sections mostly general histological staining are are used - hematoxylin and eosin, van Gieson, but special staining methods can also be used depending on the object and objectives of the study. Besides macromicroscopic dissection and histotopography, a number of other techniques are used in microsurgical anatomy studies that allow studying the structure and topography of anatomical structures in the range of optical magnification. These include microinjection methods and layer-by-layer dissection methods.

At the beginning of the XX-th century, professor V.P. Vorobiev proposed the original method of macro-microscopic examination of anatomical objects. The method was carried out using optical devices with different indication of magnification and included thin dissection of stained objects (small vessels, nerves) with their subsequent examination under a binocular loupe. This method opened a new borderline area of studying anatomical structures, but so far this technique was not used in ophthalmology, and in particular for studying the vitreous body.

In the presented works describing possible methods of investigating VB histological structure [11], the common thing was that the examination of the eyeball, including the vitreous body, was performed as a whole without distinguishing separate anatomical structures, i.e. not selectively.

The impossibility of layer-by-layer identification and histological investigation of identified VB structures could be explained by the several reasons:

- The absence of an optimal dye,
- The absence of a method to identify VB structures,
- The absence of an algorithm and method of VB dissection, which could be used to identify the vitreous body and its structures without damaging their integrity, and to perform the steps of histological processing keeping the biopsy in the initial position without damaging its structure and shape.

Thus, there are currently no approved protocols for macromicroscopic examination of the vitreous body, as well as methods for studying the vitreoretinal and vitreolenticular interface, and the improvement of VB examination methods and search for new methods remains an urgent issue.

The purpose of the study is to develop the algorithm of examination of the microsurgical anatomy vitreous body that would let to assess its topographic specifics.

Materials and Methods

Based on the results of our previous studies [12,13] we developed an original method of macromicroscopic examination of VB using Vitreocontrast imaging technique (Vitreocontrastography - a method of visualization of structures and layers of the vitreous body based on their preliminary staining. Vitreocontrast suspension has been used for the experimental studies and intraoperative visualization of the vitreous body structures since 2009. This staining agent (TU No. 9398-017-29039336-2009) is an ultradisperse suspension based on barium sulfate, insoluble in water and physiological liquids, a neutral nontoxic inorganic salt in an isotonic solution with osmolarity of 300-350 mOsm. Barium sulfate is a white crystalline substance with a molecular weight of 233.43 g/mol, particle size in Vitreocontrast suspension less than 5 microns and density of 4.4 g/cm^3. Each 1.0 ml of sterile solution contains 140 mg of dry substance (barium sulfate). Various experimental studies have confirmed the safety of intraocular administration of the suspension [14-20].

In this work during macromicroscopic examination, we used suspensions of Vitreocontrast as staining agents that meet the requirement of high adhesion. The suspensions did not allow the color of intravitreous structures change with time; allowed visualizing residual vitreous body layers and fibers on the retina and the posterior capsule of the lens with the ability to determine their precise topography, and enabled to perform thin film manipulations. A distinctive feature of macromicroscopic examination with vitreocontrastography is the possibility to isolate any structure of the vitreous body and each cortical layer to study anatomico-topographic features and relations with surrounding tissues (retinal ILM, ciliary body, lens capsule.).

For the purpose of consistent step-by-step evaluation of possible variants of vitreous topographic anatomy, an algorithm for macromicroscopic examination was developed.

Macromicroscopic ("Step-by-step") examination by dissection with application of vitreocontrast imaging technique:

- Assessment of anatomico-topographic changes of the vitreoretinal interface (by PVD induction method);
- Assessment of topographic anatomy of intravitreal structures (canals, bursae, cisterns);
- Assessment of topographic anatomy of anterior cortical layers and vitreolenticular interface.

During the macroscopic examination, it is possible to perform vitreocontrastometry with visualization of borders for precise measurement of size and topography of the stained structures and layers of the vitreous body.

The histotopographic method uses a series of histotopograms of different but precisely fixed levels. Microscopic examination of isolated structures and layers of the vitreous body:

- Isolated dissection of the structures and layers of the vitreous body;
- Fixation by the original method using a special substrate;
- Assessment of microscopic changes by an appropriate method (light or electron microscopy, immunohistochemical examination).

Making an individual map of macromicroscopic topographic anatomy of the vitreous body.

In this study, microscopic histological changes were assessed by light microscopy.

Materials and Methods

We examined 38 cadaveric eyeballs, the age of the donors ranged from 47 to 61 years, 12 were male and 26 were female, the cause of death was acute cardiovascular failure.

Eyeballs passed quality control for corneal transplantation purposes in the Eye Tissue Bank of S. Fyodorov Eye Microsurgery State Institution "EM", Moscow (Head of the Eye Tissue Bank, M.K. Khubetsova, Ph.), according to the permission for the use of medical technology FS № 2010/243 of June 24, 2010 "algorithm for the procurement of human cadaveric corneas for transplantation" and the License of the Institution of Federal State Service for Supervision of Health № 99-01-005317 from 30.04.2008 and № FS-99-01-008251 from 18.02.2013 for the type of medical activity on the intake and procurement of human cadaveric organs and tissues for transplantation.

Results

The donor's data was recorded beforehand and an individual card was filled out including indication of sex, age, occupation, cause of death, A-P axis, type of PVD, degree of VB destruction. Before corneal-scleral disc removal, the A-P axis was measured, and then, as in previous studies, the scleral petals were formed and the eye membranes were sequentially separated [12,13].

Macroscopic examination by dissection with staining of structures

Assessment of anatomico-topographic changes of the vitreoretinal interface (by PVD induction method).

Topographic and anatomical features of the vitreoretinal interface were asessed. The retina was detached from the vitreous body, thus inducing PVD (Figure 1). Staining agent was applied to the detached retinal petal and vitreous area corresponding to it (Figure 2) at an exposure time of 10 seconds. The suspension was washed off the stained surfaces with physiological solution

and macroscopic examination of the VB and its structures was performed under a Topcon OMS-800 operating microscope with ×8 to ×21 magnification indication with photo and video recording. In order to study microscopic changes in the vitreoretinal interface during induced PVD, a section of the retina as well as the VB fragment corresponding to its surface were cut off using endovitreal forceps and scissors.

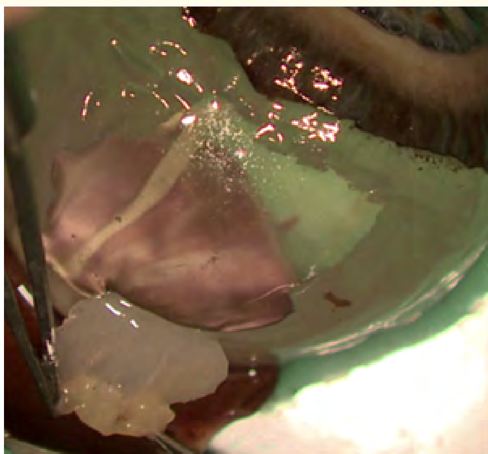


Figure 1: Separation of the retina from the vitreous body. The formation of induced PVD.

Figure 2: (a,b) Contrasting the surface of VB2 a and the corresponding areas of the retina 2b with “Vitreocontrast suspension”.

Biopsy specimens were immobilized on an Endokit (BIO-OPTICA Milano) ite adhesive-metric plate and sent for histological examination (Figure 3).

Figure 3: (a,b) Preparation and mobilization of the stained cortical layer of VB on an adhesive-metric tablet Endokit (BIO-OPTICA Milano).

Assessment of topographic anatomy of intravitreal structures (canals, bursae, cisterns).

Staining and assessment of topographic anatomy of intravitreal structures

A 30G needle was used to stain the vitreous structures. The cisterns were stained using 0.2 ml of contrasting suspension;

for antegrade staining, the needle was inserted 4 mm from the limbus, and for retrograde staining, in the posterior pole of the eye (Figure 4). The degree of structures preservation was assessed, the size of cisterns was measured, vitreous body destruction was assessed according to its degree of expression. Topcon OMS-800 operating microscope with different magnification indication was used for macroscopic examination. A suspension of barium sulfate (Vitreocontrast) was used for staining the cisterns and canals. Then, vitreous cortical layers were dissected, and the stained cisterns were cut out with microsurgical forceps and Vannas scissors, which were subsequently immobilized in a biopsy bag and placed into a biopsy cassette (Figure 5). Vitreous canals and cisterns were sent for examination by light microscopy.

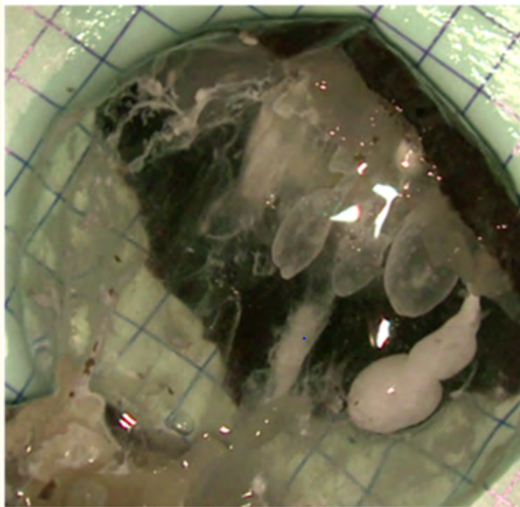
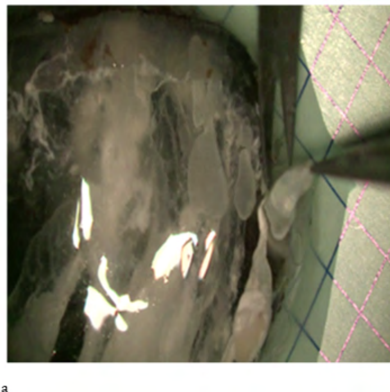
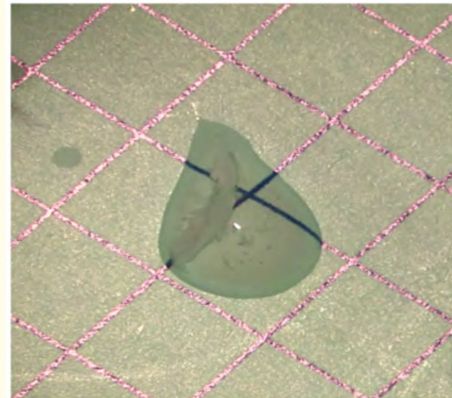


Figure 4: Stained cavities (cisterns) of the VB. Preparation on a substrate with a coordinate grid provides an additional opportunity to measure structures.





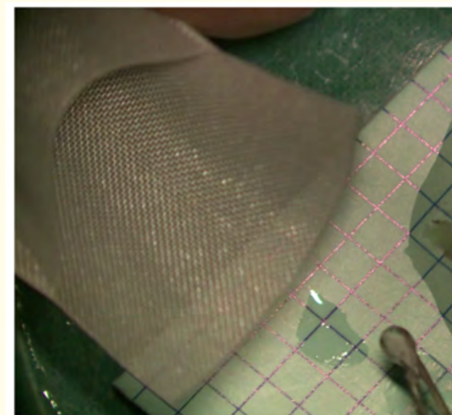


Figure 5: (a) Preparation of a contrasted VB cisterns. (b) Immobilization of the cisterns on the adhesive-metric tablet "Endokit". A substrate with a coordinate grid provides an additional opportunity to measure the size of the cisterns. (c) Placing the cisterns in a biopsy bag (BIO-OPTICA, Milano). (d) Fixing the bag with the prepared cisterns into a biopsy cassette Sacura tissue-Tek (Japan).

Assessment of the topographic anatomy of anterior cortical layers and vitreolenticular interface.

A consistent contrast of Vitreocontrast suspension of each layer of the vitreous body was performed.

The objects of this stage were sequentially stained anterior cortical layers, and the anatomico-topographic features of the vitreociliary and vitreolenticular zones were assessed (Figure 6). The relationship and preservation of anterior cortical layers and specifics of vitreolenticular interface were investigated. Vitreocontrastography dissection was performed from the posterior pole to the anterior lens capsule under retrograde investigation and from the posterior lens capsule to the posterior pole under classical investigation. To examine vitreolenticular and vitreociliary interface, Vitreocontrast suspension was successively applied onto the surface of the cortical layer (Figure 6 a,b), topographic features of the visualized layer were assessed, then using endovitreal scissors and tweezers, the layer under the investigation was selected, and separated and placed on an Endokit adhesive-metric plate or in a biopsy bag and then into a biopsy cassette.

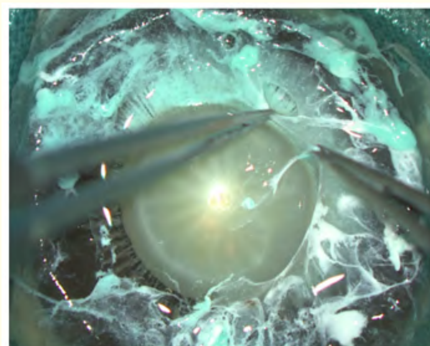


Figure 6 (a,b): A view of anterior cortical layers, vitreolenticular and vitreociliary interfaces before contrast b view after contrasting with Vitreocontrast suspension.

Successive layer-by-layer contrasting and dissection of the anterior layers of the vitreous body is performed 6 (c, d, e, f).

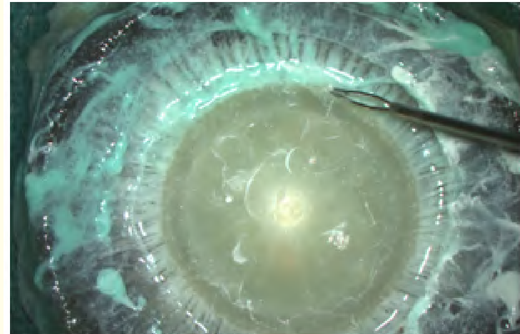


Figure 6 (c): Preparation of the anterior cortical layer. The contrasting layer of the vitreous body is indicated.

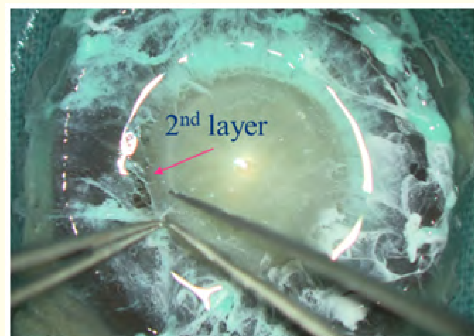


Figure 6 (d): Sequential dissection of the contrasted anterior layers of the vitreous body.

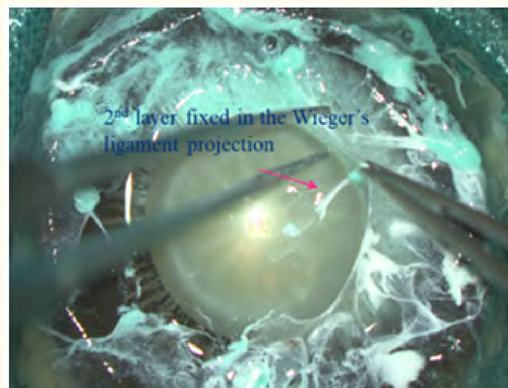


Figure 6 (e): Then the next vitreous cortical layer was stained thus performing lamellar dissection of each vitreous cortical layer defining its topographic peculiarities

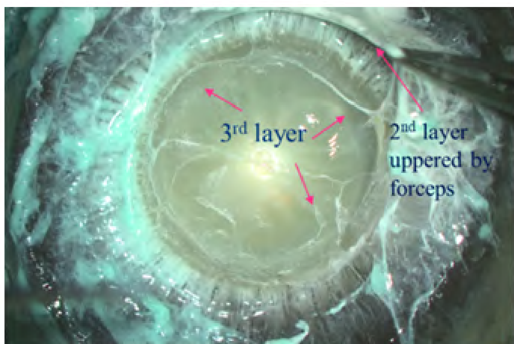


Figure 6 (f): Dissection of the next contrasted anterior cortical layer. It is possible to visualize each contrasting layer of the vitreous body with the study of its topography.

Each layer of the anterior cortical layers of the vitreous body can be sequentially separated. Biopsy specimens were immobilized on an Endokit (BIO-OPTICA Milano) ite adhesive-metric plate and sent for histological examination.

Investigation of the interposition of CT and Zinn ligaments, their safety, length, attachment points Figure 7 (a, b, c, d).

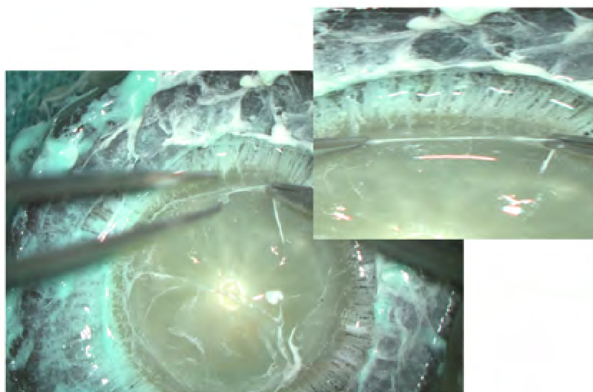


Figure 7 (a): A portion of the Zinn ligaments is fixed to the anterior cortical layer of the vitreous body.

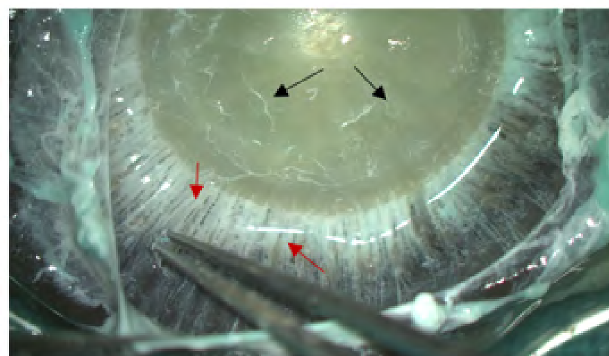


Figure 7 (b): The contrasted zinc ligaments (red arrow) and a thin layer of vitreous body on the posterior capsule of the lens (black arrow) are visualized.

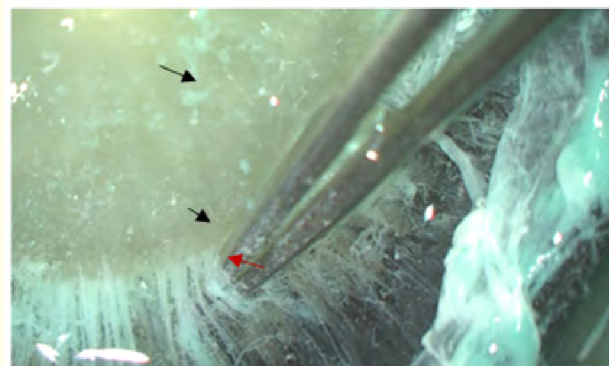


Figure 7 (c): A portion of zinc ligaments (red arrow) fixed to the posterior capsule of the lens and the cortical layer. The vitreous layer is visualized in the posterior capsule of the lens (black arrow).

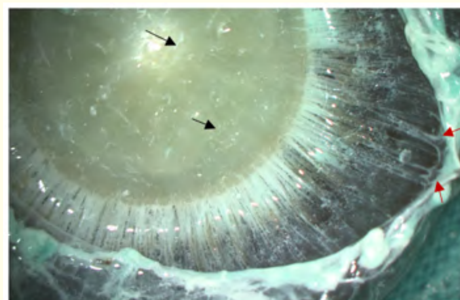


Figure 8: The study of topographic anatomy vitreolenticular and vitreociliare interface. Renders a layer of the vitreous body on the lens surface (black arrow), contrasting of the Zinn ligament, fixed to the base of the vitreous body (red arrow).

It should be noted that in all cases, the vitreous layer contrasted on the posterior capsule of the lens, which suggests the presence of a retrolental bag, and not the space between the capsule of the lens and the anterior cortical layers [13,16].

The histotopographic method uses a series of histotopograms of different but precisely fixed levels.

For histological examination, the structures of VB were fixed in a 10% solution of neutral formalin, washed with running water, dehydrated in alcohols of ascending concentration and poured into paraffin, a series of histological sections were performed using hematoxylin-eosin staining. After the preparation of the glass preparations, a microscopic examination was performed under a Leica DM LB2 microscope at ×50, ×100, ×200, ×400 multiple magnification followed by photographing.

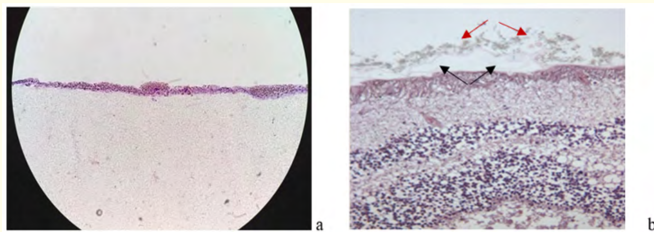


Figure 9 (a,b): a Histological preparation of the cortical layers of the vitreous body of the donor eyeball on a substrate. Color hematoxylin-eosin, ×630. b Corresponding area fragments of the vitreoretinal interface, split cortical layers of the VB (black arrow) with adhered particles of Vitreocontrast suspension (red arrow). Abnormal posterior vitreous detachment. Color hematoxylin-eosin.

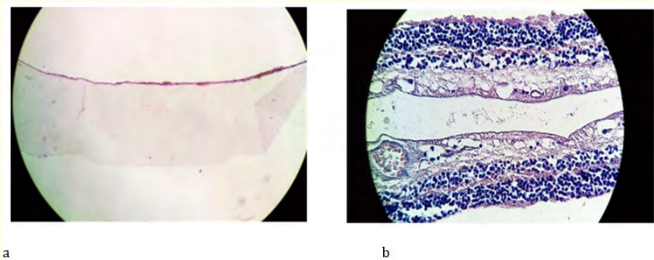


Figure 10 (a,b): a Histological preparation of the cortical layers of the vitreous body of the donor eyeball on a substrate. Color hematoxylin-eosin, ×630. b Histological preparations of the retina of the donor eye without a vitreous body on the inner surface. Normal posterior vitreous detachment. Color hematoxylin-eosin, a ×400; b ×630.

Drawing up an individual map of the topographic anatomy of vitreous structures with photo and video registration.

Discussion

The method examination of the microsurgical anatomy vitreous body including original dissection technique

«Step by step» and consistent dissection algorithm enabled to consistently step—by-step distinguish isolated vitreous structures, to study their morphology and to keep the form and the integrity of the preparations after all the stages of histological processing including making of sections and staining for light microscopy. The specifics of VB histological investigation is the fact that it is impossible to fix it because of the size of the specimens and the number of the quantity of the material contained in them. To fix VB specimens we used the method with the fixation of separate VB structures on a special adhesive-metric plate that we placed into the biopsy bag and then into the biopsy cassette. Then the isolated preparations of VB structures were fixed into formalin and delivered to the laboratory where standard processing took place. This method allows receiving new data about VB spatial anatomy and histology as well as to revealing the nature of the vitreous pathological changes in different pathologies. The developed method of macromicroscoping examination of the vitreous makes it possible to make an individual map of the vitreous topographic anatomy and may be proposed for the vitreous body investigation.

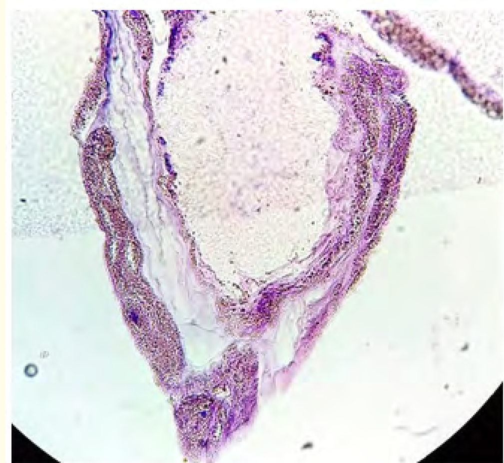


Figure 11: Histological preparation of the vitreous cisterns of the donor eyeball on a substrate. Color of hematoxylin-eosin, ×200.

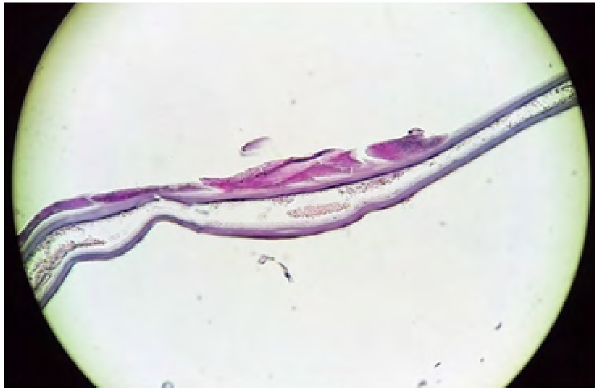


Figure 12: Histological preparation of the anterior cortical layers of the vitreous body of the donor eyeball on a substrate. Color hematoxylin-eosin, ×200.

Conclusion

The examination of the microsurgical anatomy vitreous body step-by-step by dissection method with the use of Vitreocontrast imaging technique allows fully assess the topographic anatomic picture of VB structures, vitreoretinal and vitreorenticular interface and perform full microscopic histological investigation separately distinguished structure or layer of the vitreous body with a possibility to assess received specimen by light microscopy.

Macromicroscopic investigation Step-by-step by dissection method with the use of Vitreocontrast imaging technique allows for the full assessment of spatial topographic anatomic picture of the structures of the vitreous body, vitreoretinal and vitreolenticular interface and making full microscopic histological investigation of the separately distinguished structure or layer of the vitreous body with a possibility of assessing the obtained specimens by light microscopy.

The developed algorithm examination of the microsurgical anatomy vitreous body allows making an individual map of the vitreous body topographic specifics and can be offered for the investigation of the vitreous body. On gathering sufficient material and completion of its statistical processing it is possible to present the maps of topographic anatomy in normal, age-related and pathological conditions of the vitreous body.

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16. NM Kislitsyna., *et al*. Anatomic-Topographic Features of the Anterior Cortical Layers of the Vitreous Body Fyodorov's Eye Microsurgery Complex, Moscow, Russia Citation: NM Kislitsyna., et al. Anatomic-Topographic Features of the Anterior Cortical Layers of the Vitreous Body. *EC Ophthalmology* 10.12 (2019): 01-10.

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