ПОСТРОЕНИЕ КОМПЬЮТЕРНОЙ МОДЕЛИ ПРИ МОРФОМЕТРИИ ТКАНЕЙ

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При исследовании тканей животных и человека под микроскопом исследователи сталкиваются с проблемой их объективной оценки. Описательная микроскопия является субъективной и не позволяет сделать точных выводов. Частичный подсчет выборочных элементов ткани часто недостаточно информативен. Использование полноценной морфометрии является крайне трудоемкой процедурой, которую сложно провести, повторить и перепроверить. Применение описательной микроскопии не позволяет построить модель исследования для всесторонней оценки полученных результатов, что затрудняет выводы. ***Цель.*** Решить проблему объективной оценки состояния тканей при гистоморфологических исследованиях и ускорить их выполнение с помощью компьютерного моделирования. ***Материалы и методы.*** Весь процесс от получения микропрепарата исследуемой ткани до окончания его полноценного анализа был разделен на 4 этапа: 1) фотографирование всей площади микропрепаратов с помощью видеоокуляра микроскопа, 2) подсчет гистоморфологических элементов на фотографиях, 3) построение компьютерной модели, 4) анализ полученных данных. ***Результаты.*** Нами была построена интерактивная компьютерная модель эксперимента, в которой все параметры были связаны в единую совокупность, и изменение любого значения влияло на всю модель. В ней можно визуализировать полученные результаты, рассчитывать новые параметры, находить взаимосвязи между ними и использовать дополнительные инструменты, например, использовать машинное обучение для поиска неочевидных взаимосвязей между компонентами или ускорения дальнейших расчетов. ***Выводы.*** Достоинствами компьютерного моделирования является то, что оно существенно ускоряет проведение гистоморфологических исследований, улучшает качество их обработки, делает их более объективными и дает ученым больше возможностей для глубокого анализа. Важным преимуществом компьютерного моделирования является то, что оно может использоваться для любых гистоморфологических исследований.

Ключевые слова: *морфометрия, компьютерное моделирование, кистозная мастопатия, гистоморфологические исследования, молочные железы.*

CONSTRUCTION OF COMPUTER MODEL IN TISSUE MORPHOMETRY

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Background. In a microscopic study of animal and human tissues, researchers are faced with the problem of their objective assessment. Descriptive microscopy is subjective and does not allow to make exact conclusions. A partial counting of some selective elements of tissue is often not sufficiently informative. Use of high-grade morphometry is a very laborious procedure, which is difficult

to conduct, repeat and recheck. Descriptive microscopy does not allow to make a model of research for a comprehensive assessment of the results, which complicates making conclusions. *Aim*. To solve the problem of objective assessment of tissue condition in histomorphological studies and accelerate their implementation with the help of computer modeling. ***Materials and Methods.*** The whole process from making micropreparations to the end of their full analysis was divided into 4 stages: photographing the entire area of micropreparations using a video eyepiece microscope, counting histomorphological elements in the photos, construction of a computer model, analysis of the obtained data. ***Results***. An interactive computer model of the experiment was constructed, in which all parameters were combined into a single set, and a change of any value influenced the entire model. It was possible to visualize the obtained results, calculate new parameters, find out the relationship between them and to use additional tools, as, for example, machine training for finding non-obvious relationships between components or for speeding up further calculations. ***Conclusions.*** The advantages of computer modeling consist in that it significantly accelerates histomorphological examinations, improves the quality of their processing, makes the procedure more transparent and provides scientists with more opportunities for in-depth analysis. An important advantage is that this technique is suitable for any histomorphological studies.

Keywords: *morphometry, computer modeling, cystic breast, histomorphological studies, mammary glands.*

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Materials and Methods

Morphometry of tissues is usually conducted by direct microscopy [1-3]. All tissue elements are counted in the microscope field which is moved by a researcher «by eye's estimation» with inevitable overlapping of fields which significantly influences the results. It is rarely possible to look through all microscope fields due to a high labor intensity of the process.

The surface area of the studied elements is not calculated or is assessed approximately with use of a reticle eyepiece. The section area of the entire preparation is calculated by application of millimeter paper onto the micro-preparation. In this case it is impossible to distinguish between different kinds of tissues (for example, between muscle tissue and stroma), and a significant error exists.

With manual microscopy and analysis, processing of one micropreparation takes up to 4-6 hours. Here, one cannot exclude mistakes that are difficult to detect. Correction of mistakes requires time comparable with that used for the primary analysis of micropreparations [1].

Aim of work: partial automatization of histomorphological method for objective assessment of the condition of the studied tissues and for acceleration of histomorphological examinations.

We examined mammary glands of normal rats and of rats with cystic breast [2,4]. Morphometric analysis of 253 micropreparations of rats' mammary glands was conducted by 23 parameters [3].

I. Taking photos of micropreparations.

Micromed-2 microscope with x40 magnification was used. Photos of micropreparations were taken using video eyepiece for Toup Cam 9.0 mp microscope (resolution 9 megapixels). It appeared to have excessive resolution while for the required quality of photos 3 megapixel resolution was sufficient. Here, photos partially overlapped. This did not influence the final model since all the basic parameters were calculated relative to the total area of photos and not to the area of the section of the micropreparation. There were made 10-30 photos of each micropreparation which took about 2-3 minutes. To study 253 micropreparations, in total about 5,000 photos were taken which required about 12 hours.

II. Counting of tissue elements on photographs.

The obtained histomorphological photos were downloaded into a graphic editor (for example, Photoshop) and were processed layer-by-layer with calculation of the required param-

eters. For example, at the first stage everything not related to the micropreparation was eliminated, and the area of the whole tissue was calculated, at the second stage everything not related to stroma was eliminated with calculation of the area of stroma, and so on. The area of remained or eliminated elements of tissue in pixels (px) could be viewed in the extended «Histogram» window (shows the area of the highlighted figure). Besides the area, any other elements could be counted, for example, ducts of mammary glands or vessels. All calculated areas and values were recorded in Excel table.

This simplified method of computer morphometry resembles manual morphometry, but it is much faster and more convenient, since the calculation is carried out on a large screen of a computer with the probability for emphasizing the elements and for application of filters which is impossible with direct microscopy of the examined tissues.

III. Construction of computer model.

All values of parameters for each photo of a micropreparation were summated («summation» function), and final values for each micropreparation were obtained.

After that calculations were made using formulas (taken from the section «formulas» or written by the researchers themselves).

The areas of histomorphological elements were converted from px to μm^2. Resolution of the studied data was 800/600 px. With x40 magnification of the microscope: 1 mm=275 px, 1 mm^2=75,625 px, 1 $\mu m^2 \approx 0.0746$ px, 1 px$\approx$13.22 μm^2.

The area of pixels was calculated using the formula

$$1\,px = r\frac{S1}{S2}$$

where r is pixel resolution of a certain photoin μm^2, S1 – surface area of a certain photo, S2 – surface area of a new photo.

Accordingly, with resolution 872/654 px: $1\,px = 13.22 * \frac{800*600}{872*654} \approx 11.12$ μm^2. If magnification is changed, the value is multiplied by the ratio of the previous magnification to a new one.

Then a scheme was constructed in which all values were subject to a series of mathematical transformations to obtain the values of each parameter. This is a mathematical model of the conducted experiment that was presented in Excel in the form of successive columns in each of which a specific parameter was calculated. With this, at any moment it is possible to take any value from any column and use it for calculations (Fig. 1).
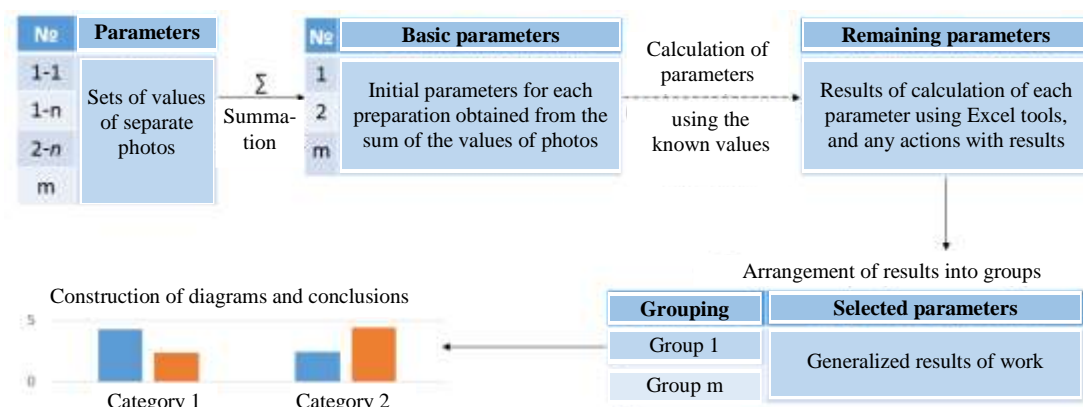

Fig. 1. Algorithm of construction of computer model in Excel

If in the experiment several groups of objects (for example, of animals) are studied which should be differentiated against each other, an additional stage is included to find the average values for each group with automatic calculation of mean square deviation.

In our case 23 groups of experimental animals were studied, therefore the average parameters were calculated for each group. On the basis of the average values for each group conclusions were made for the results of the work.

In the experiment 23 morphometric parameters were calculated:

1) AHS (mm^2) – area of histotopographic section of mammary gland.

2) Q (in pieces) – total quantity of ducts in a section.

3) QS (in pieces) – quantity of single ducts in the maximal cross section of mammary gland.

4) QDG (in pieces) – quantity of ducts arranged in groups.

5) QG (in pieces) – quantity of groups of ducts.

6) P (in pieces per mm^2) – density of glandular field = Q/AHS.

7) DG – density of groups of ducts, equals QG/AHS.

8) ADG – average quantity of ducts in one group, equals QDG/QG.

9) %S – percentage of single ducts, equals QS/Q·100%.

10) %G – percentage of grouped ducts of all ducts = 100%-%S.

11) %AGF – percentage of area of glandular field, equals percentage of the area of ducts of the area of mammary gland tissue.

12) %AS – percentage of the area of stroma, equals 100% - % of the area of ducts.

13) AGF – area of glandular field in mm^2, equals AHS·%AS.

14) AS – area of stroma in mm^2, equals AHS – AGF.

15) DV – degree of vascularization, equals the quantity of vessels/AS.

16) ADD – average dimension of one duct, equals AGF/Q in μm^2.

17) CS – cellularity of periductal stroma.

18) %C – percentage of cells.

19) HE (in μm) – heigh to fepithelium. Assessment of the height of epithelium (thickness around ducts) in μm.

20) AADE (in thousands of μm^2) – average area of ductal epithelium.

21) ADL (in thousands of μm^2) – average area of a duct lumen, equals ADD-AADE.

22) ADEM (in thousands of μm^2) – area of ductal epithelium in maximal section, equals AADE·Q.

23) AGL (in thousands of μm^2) – area of glandular lumen, equals AGF-ADEM.

It should be noted that parameters P, ADD, ADL are considered the most important for assessment of a degree of cystic breast.

Results and Discussion

For calculation of practically all morphometric parameters 6 basic parameters are required: area of section, area of stroma, area of glandular tissue, area of lumens, quantity of ducts, quantity of vessels. Earlier the basic parameters (ADD, ADL) were calculated by indirect methods using formulas, with a high degree of error. Application of computer morphometry permitted to significantly improve accuracy of calculation of these parameters.

Visualization and assessment of relationships between the studied parameters permitted to detect some mathematical inaccuracies of the morphometry of mammary glands that was difficult to detect earlier without computer modeling. In particular, it appeared that division by the quantity of ducts often gives false-high and false-low results. This is due to the fact that ducts of mammary glands normally are practically invisible, and in result the number of ducts (denominator in many above described parameters) appears very low which makes the final result higher. And, vice versa, in case of prominent cystic breast all ducts are visible which makes the results false-low in comparison with partial regression of cysts when the total area of ducts practically remains unchanged, but minimally dilated ducts become invisible.

In our opinion, for more adequate evaluation of the condition of mammary gland tissue the following parameter may be used which will be especially effective in assessment of the extent of evidence of cystic breast condition: %DD (degree of dilation of lumens of ducts relative to the area of stroma). It equals ADL/AS. This parameter is helpful in the general assessment of the condition of ducts irrespective of the quantity of ducts (Q). Here it is possible to see what part of stroma is taken by dilated ducts which vividly reflects the extent of evidence of cystic breast.

With manual morphometry, it is very difficult to calculate this parameter without high error.

After having obtained a computer model in Excel further research is conducted, in particular:

1. Statistical processing of results using Student's and other methods (exist in Excel).

2. Visualization of results of work in graphic charts and diagrams.

3. Use of the obtained values at any stage for additional research activities (for example, use of machine training tools for establishment of relationships between the studied morphometric parameters).

4. Calculation of new parameters required for improvement of the model or of the method in general.

In our opinion, the advantages of a computer model are:

1. The given model is suitable for analysis of any tissue and not only of mammary glands.

2. All results are in the same place, and it is possible to visualize any parameter. This permits to see the results of the work in whole which facilitates formulation of conclusions and search for errors.

3. In case separate values or formulas are changed, the whole model is immediately recalculated which considerably accelerates research work.

4. The results may be checked at any level of research which simplifies search formistakes and improves transparency of the procedure of the scientific work.

5. The speed of morphometry is approximately 2-3 times that of direct micros-copy even without use of machine training methods.

6. The obtained data may be used to establish generalities including those for construction of combined models to be used for description of the processes occurring in the organism, from different points of view.

The main conditions for creation of a computer model are possessing certain experience in using such programs as Photoshop and Excel (or their analogs) and availability of video-eyepiece for a microscope.

Computer modeling in morphometry of tissues that was carried out in cystic breast condition, may be as well used in other diseases of mammary glands [5] and in investigations of other organs and tissues.

Conclusions

1. Computer modeling in morphometry of tissues provides many possibilities for fast conduction of high-quality scientific work.

2. Computer modeling has a number of advantages over direct microscopy in analysis of the results of histomorphological examination of tissues.

3. In future, construction and improvement of computer models will permit to create combined models that could be usedfor description of functioning of an organism at different levels of its organization from different positions simultaneously.

4. For a more precise assessment of the condition of the mammary gland tissue a new parameter is proposed: (%DD) – the degree of dilation of ducts which effectively shows the condition of the mammary gland tissue in cystic breast condition.

Литература

1. Автандилов Г.Г. Медицинская морфометрия. Руководство. М.: Медицина; 1990.
2. Мустафин Ч.Н., Кузнецова С.В. Дисгормональные болезни молочной железы. Клиническое руководство. М.: Медицина; 2009.
3. Чумаченко П.А., Шлыков И.П. Молочная железа: морфометрический анализ. Воронеж: Издательство Воронежского государственного университета; 1991.
4. Васин А.С., Давыдов В.В., Свирина Ж.А. Исследование влияния релиз-активных препаратов на фиброзно-кистозную мастопатию // Фундаментальные аспекты психического здоровья. 2017. №2. С. 156-159.
5. Мнихович М.В. Эпителиальный и стромальный компоненты при протоковом раке молочной железы. Российский медико-биологический вестник имени академика И.П. Павлова. 2015. Т. 23, №3. С. 99-105. doi:10.17816/PAVLOVJ2015399-105

References

1. Avtandilov GG. *Meditsinskaya morfometriya. Rukovodstvo.* Moscow: Meditsina; 1990. (In Russ).

2. Mustafin CN, Kuznetsova SV. *Disgormonal'nye bolezni molochnoy zhelezy.* Moscow: Meditsina; 2009. (In Russ).

3. Chumachenko PA, Shlykov IP. *Molochnaya zheleza: morfometricheskiy analiz.* Voronezh: Izdatel'stvo Voronezhskogo gosudarstvennogo universi-teta; 1991. (In Russ).

4. Vasin AS, Davidov VV, Svirina JA. Study of the effect of release-active drugs on fibrocystic breast disease. *Fundamental'nye aspekty psikhicheskogo zdorov'ya.* 2017;2:156-9. (In Russ).

5. Mnikhovich MV. Epithelial and stromal components in ductal brest cancer. *IP Pavlov Russian Medical Biological Herald.* 2015;23(3):99-105. (In Russ). doi:10.17816/PAVLOVJ2015399-105

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