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MORPHOLOGICAL AND IMMUNOGISTOCHEMICAL METHODS IN ALIMENTARY IRON DEFICIENCY OF THE THYMUS OF WHITE RATS INDICATORS

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Abstract
In this study, morphological and immunohistochemical changes in the lymphoid structures of the thymus of white outbred rats with alimentary iron deficiency were studied. Histological analyses revealed a violation of parenchymal-stromal balance in the organ, a decrease in morphometric indicators of lymphoid tissue, the number of lymphoid cells, and the level of expression of immunohistochemical markers. In immunohistochemical studies, a significant decrease in the expression of markers CD3, Ki-67, and Bcl-2 was noted, which indicates a negative effect of iron deficiency on the proliferation, differentiation, and immunocompetence of T-lymphocytes. The research results have scientific and practical significance in the in-depth analysis of iron deficiency-associated immunodeficiency states and the development of strategies for their correction.

Keywords: Iron deficiency, thymus, white rat, lymphopoiesis, T-lymphocytes, immune system.

Introduction

The actuality

To maintain the health of the human immune system plays an important role. Thymus is a member of the central immunogenezning. It not only at, but also by the members is also immune peripheral t - lymphocytes and tabaqalanisnini provides the immune reaction at the same time for the implementation of administrative and t-lymphocytes and stimulates the integration of various populations macrofags [1, 3, 4, 5, 7].

The lack of micronutrients in the human body resistant to the suppression of unfavorable environmental factors of the body, going beyond the limit of the formation of an immune deficiency state, the activity of the antioxidant defense system of the disorder, the development of the form of chronic diseases, increase the risk of developing common diseases, quality of life and effective treatment measures will lead to a decline. The lack of important micronutrients leads to a violation of the metabolic process, reduces the body's resistance to infections and various other pathogenic factors [2]. Iron (fe) is an essential element for all living cells, because in many metabolic processes, including dna synthesis and transkripsiya, is involved in the transportation of oxygen [6].

In turn, the immune reaction of the body in proportion to maintain the optimum balance of micronutrients and provides protection from viral infections. For this reason it is vital, especially

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zinc, selenium, iron, magnesium shortage bioelementlarning member and like many pathological process that will create the system to the ground in the night [8].

The purpose of the research the features of the thymus of rats alimentar yetishmovchiligida white iron consists of the evaluation of morphological and immunogistokimyoviy.

Material and Methods

Research was conducted in male rats than 80 without white seed. The rules of ethics on the use of animals in experimentation, Xelsinki the requirements of the congress will follow. Rats were kept in viva simple conditions. Experience in the laboratory of the animal's age, sex, weight, nutrition were kept correspond to the conditions in the environment. Thymus structure morfofunktsinal two indicators to determine a group of animals was established. I group - normative (n=40); group ii – rats given magnesium reached white diet (n=40). For modeling a shortage of micronutrients German "ALTROMIN Spezialfutter

Gmbh & Co. KG" prepared by the firm led to the use of a special feed. Feed

N 36/2024 have been provided with an official certificate special number. 2 sticks per day to rats in the control group were given the usual feed. Without special body weight in feed per day due to the experience of the group 2 sticks of 20 g were used.

The experimental and control groups was removed from the experiment the rats under anesthesia without the white seed was dekapitatsiya and air. Open the chest and the thymus were separated. The portion of the thymus tissue in 10% formalin in fiksatsiya neytrallash am and after being washed in running water for 2-4 hours, which increased in concentration and spirtlar xloroform suvsizlantirildi, the wax block was prepared in accordance with generally accepted methods. Mkm wax blocks 4-6 thick cut down gematoksilin – eozin and van for your business, the method was painted. Thymus structure struktur okulyar of the drug-using, check mikrometr morfometrik was. The thickness of the different parts of the capsule of the thymus, trabekula diameter, shunihgdek, thymus fragments, kernels bark and the relative area of the floor (in relation to the total area of the incision), the bark will measure the width of the floor. Each of the five histological measurements in the view area of the incision was carried out. View of the area were selected on a random basis.

In order to study the structure of the cells of thymus limfoid, NOVELLA NLCD Model-

307 (China) using a microscope, pitches immersiya under structural qismalarida of the thymus (subkapsulyar, kort the floor of the fields and kernels)on the number of cells was counted. Okulyar installed to count the number of cells was carried out using a microscope to morfometrik November. Immunogistokimyoviy drugs (3-4 lived in mkm thick) Ben MarkXT Ventana (Switzerland) avtosteynerida was prepared. The process gistotopogra-CD3 fiy computer's study, K-67 and bc cml-2 sensitive to the 1:100 diluted at a ratio of poliklonal antitanalar (Ventana, switzerland) were used in the case of roofing adhesive histological oynachalarida items (Ventana, Switzerland) was carried out. Immunohistokimyoviy antitanalar reactions was conducted according to manufacturer's protocol. The result of the reaction were assessed by counting cells stained positive 10x100 see enlarged ten times and results are expressed in percent was in the area.

Obtained during the research of morphological, morfometrik immunogistokimyoviy mathematical data processing and Pentium – IV software package on a personal computer with microsoft office

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Excel "7,0" matrisa was carried out at the general's. Thus, the standard deviation and reprezentativ will identify errors.

Digital information variatsion pumps was established in arifmetik average deviation, thus the average error and percent deviation from the control of the size variatsiya coefficient will be calculated. The statistical significance of deviations of the results obtained from the appropriate control method for comparison of two independent samples parametrik - Styudent eye (in the mode of the normal distribution) were assessed using. Differences of $p \leq 0.05$ statistical significance is that it has a value of up to. Issued to the organization of research in evidence-based medicine principles will apply.

Results and Analysis

Paired with one of laboratory animals of the thymus in the control group consists of two pieces, if you make it a part of to'sh in the lower third of the bone is located. 6 and 9 month healthy white seed capsule of the thymus of rats, respectively, the thickness of the gate without the field 5,82±0,32 and 5,97±mkm was of 0.38. The diameter of the proximal part Trabekulaning respectively 13,36±0,27 and 13,52±0,22 mkm, in the distal portion 10,28±0,14 and 10,43±0,22 determines that it is equal to mkm. Thymus area corresponding to the period of the age of the piece without 64,27±0,12% and 52,27 ± 0,48% accounted for.

I am modeling the thymus of rats in the group without iron deficiency breed the white gate white rats in the control group, the thickness of the capsule of the field in comparison with the 6 young 1,05 times the monthly period, 9-month period increased to young 1,06 times. The diameter of both the proximal and distal part of the age of trabekulaning due to the period without 3,9% and 4,7% increase will determine. The 6 month period piece of the area of the thymus in young 1,04 times, 9 times, respectively, and decreased to month period 61,89 young 1,06±0,52% and 49,53 ±0,63% accounted for.

Healthy white seed kernels and thymus of rats of the portion of the tissue without histological prepretlarida po'sloq is the difference of the floor. Po'sloq kernels of the border between the exact expression and the floor was not. Parenximasi will identify the piece was replaced by fat tissue. The bark of the thymus in the control group 6 and 9 respectively of the floor area of laboratory animals monthly 63,86±0,37% and 58,83±0,26%, respectively, of the floor area 28,52 kernels±of 0.38% and 32,92±0,42 % accounted for (fig.1).

Bark – the age of the index during the period of both kernels without becoming 2,24±0,16%, and 1,78±0,36% determines that it is equal to. The thickness of the bark layer of the monthly period in young 248,27 6±11,54 mkm, in the period of 9 months young 165,27±9,76 was to mkm.

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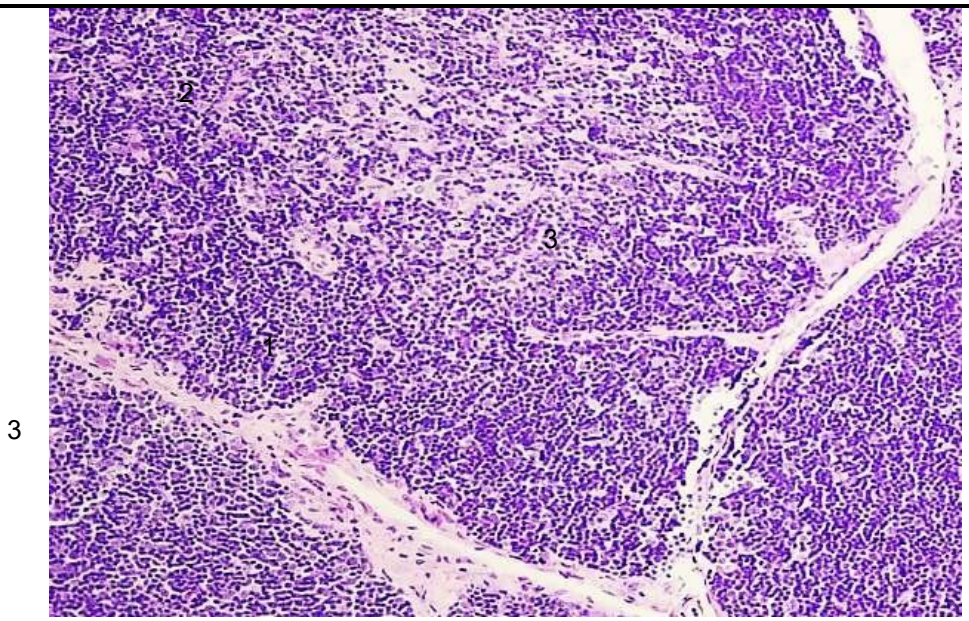

The picture.1. In the control group, 9-white monthly of the thymus of rats. Gematoksilin -painted with eozin. Ok. 10 x weather. 10. 1-the bark floor, 2-floor kernels, 3-trabekula.

I am iron shortage modeling of the thymus of rats in the group without the bark of the white breed of white rats in comparison with 6-month age period intakt floor area of 4.2 %, 9 month age period 5,8% and decreased by. Both kernels 1,03 1,05 times the floor area is reduced since the age of and during the period of, respectively, were detected (fig.2). Bark – index both decreased, respectively, during the period of the age of the kernels 2,23±0,11% and 1,77±0,18% accounted for. Healthy white kalamshlar indicators in comparison with the thickness of the floor of the bark, 6 and 9 months of age, respectively, in the period decreased to 1,05 times.

Healthy 6-month age period of laboratory animals of the thymus t-lymphocytes analyzed by a particular type when the amount of the floor of the bark in the field of small lymphocytes subkapsulyar 38,42±0,58%, 64,78 area kortiral+0,44%, kernels on the floor 34,28± 0,17% accounted for. The average amount of lymphocytes in the field of the floor of the bark subkapsulyar 17,58±0,26%, kortiral area 16,37±0,22%, kernels on the floor 31,26±0,18% is equal to. The floor of the bark subkapsulyar the amount of large lymphocytes in the field 17,64±0,28%, kortiral area 6,32±0,12%, kernels on the floor 4,26±0,10% accounted for.

9 month age period in the thymus of rats in the control group without the bark of the white breed of small lymphocytes in the field of the amount of the floor subkapsulyar 33,46±by 0.41%, kortiral area 52,92+0,36%, kernels on the floor 26,12± 0,14% accounted for. The average amount of lymphocytes in the field of the floor of the bark subkapsulyar 12,73±0,18%, kortiral area 11,94±0,16%, kernels on the floor - 32,27±0,22% is equal to. The amount of the floor of the bark in the field of large lymphocytes subkapsulyar 12,68±0,14%, kortiral area 4,26± 0,10%, kernels on the floor 3,19±0,16% accounted for.

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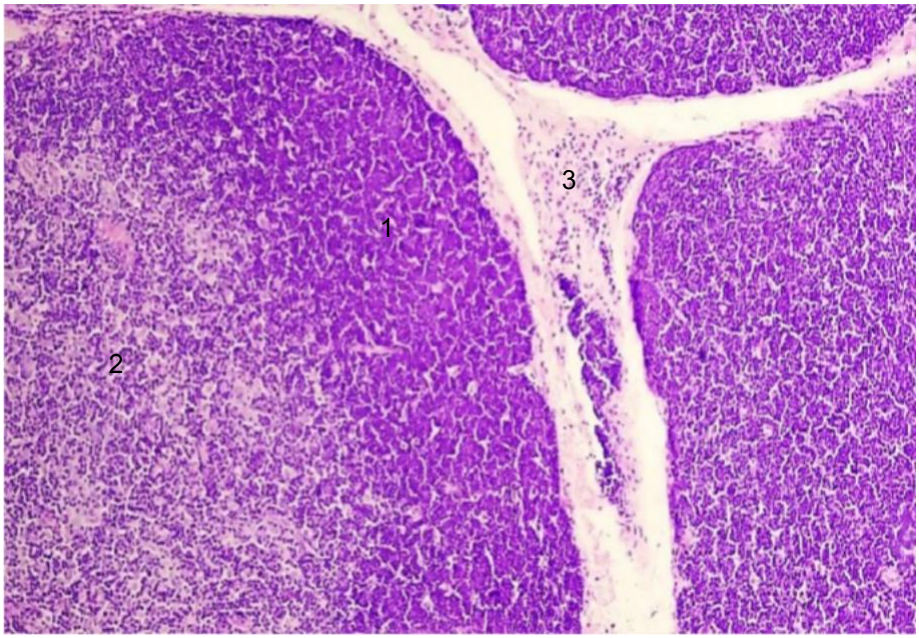

The picture. 2. Fear of failure in the group Alimentar 9-white monthly of the thymus of rats. Gematoksilin -eozin toe with'not only be spiritually healthy. Ok. 10 x weather. 10. 1-reduced the floor area of bark, 2-kernels increased floor area, 3-trabekula thickened.

Seed the thymus of rats in the group called white iron deficiency without 6 month corresponding to the amount of the components of small lymphocytes without 4,0%, 5,0% and 4,0%, and medium lymphocytes by the amount of 2.0%, 2.1% and 4,1%, and the amount of large lymphocytes 4,0% increase of 1.3% and 1,0% and decreased by. 9 seed the thymus of rats in the experimental group corresponding to the components of the monthly white without case, a small amount of lymphocytes 5,1%, compared with 6.1% and 5,0%, and medium lymphocytes and the amount of 3,0%, manuals of 3.1% and 4.2%, and the amount of large lymphocytes 5,0% and 1.0 percent, respectively, to determine the reduction.

Analysis of thymus cell reactions immunohistokimyoviy gave me the opportunity to determine the content of the piece, and the bark-t lymphocyte marker cd3 in the field of representing kernels showed a positive reaction to give. The white bark and seed kernels of the thymus of rats for 6 months without the amount of CD3 positive cells in the floor fit without 45,68±1,36% and 27,83±0,62%, in the period of 9 months in laboratory animals yosin fit without 35,46±1,08% and 24,27±0,36% accounted for.

Healthy white cells that seed the thymus of rats without drug of representing Or-

Special treatment was given for the sake of determining 67 markyorini antitelo proliferatsiya and many many cells will identify with. 6 monthly period without the share of these cells in rats breed yosin the white bark on the floor 36,12±1,02%, kernels on the floor 12,74±0,68%, in the period of 9 months in laboratory animals iosyn fit without 25,92±is 0.74% and 7,09±0,48 % is equal to.

Anti-apoptotik protein - cml bc-2 in the parts and pieces of bark to the positive reaction you will ma'n member, as well as kortikomedulyar observed in the cell area, and 6 and 9 months of age, respectively, in the period 8,16±0,16% and 10,94±0,18% accounted for.

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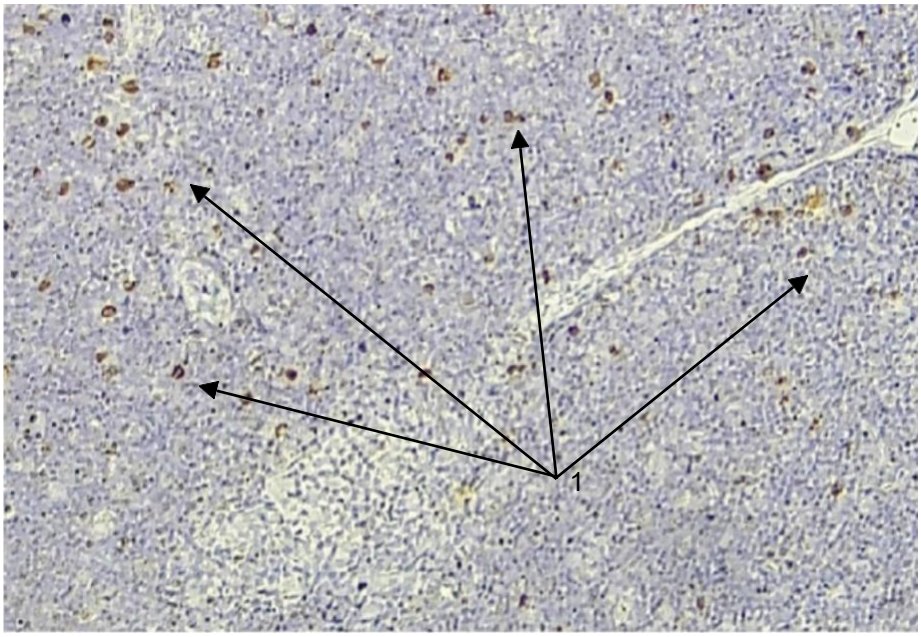

Rasm.3. Alimentar Fe yetishmovchiligi guruhidagi 9-oylik oq kalamush timusi. Ki- 67 markyoriga maxsus antitela bilan immunogistokimyoviy reaksiya. Ок. 10. Об. 10. 1 – Ki-67 -musbat hujayralar. Ekspressiya darajasi po'stloq qavatda 20,89% va mag'iz qavatda 4,14%.

In white outbred rats with iron deficiency, at 6 months of age, the level of expression of the CD3 marker decreased by 5.0% and 4.1%, respectively, for the components of the thymus, and by 6.1 and 5.0%, respectively, at 9 months of age. The level of expression of the Ki-67 marker decreased by 3.8 and 2.0%, respectively, at 6 months of age, and by 5.0 and 3.0%, respectively, at 9 months of age (Fig. 3). The level of expression of the anti-apoptotic protein - bcl-2 decreased by 2.0% and 3.1%, respectively, by age periods.

Conclusion

The study found that iron deficiency has a significant negative effect on the thymus of white outbred rats. With iron deficiency, the thymus capsule thickens, the diameter of the trabecula increases, the area of the organ lobules, the thickness of the cortex, and the corticospinal index decrease in all age periods. A decrease in the proportion of small, medium, and large forms of T-lymphocytes, as well as CD3 marker-positive cells, indicates a slowdown in the maturation of T-lymphocytes against the background of iron deficiency. A significant decrease in the number of Ki-67 positive cells, representing lymphocyte proliferation, indicates a decrease in regenerative activity, and low expression of the anti-apoptotic protein Bcl-2 indicates an increase in apoptosis processes in the thymus. This indicates a decrease in the functional activity of the thymus in iron deficiency.

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