The aim of this work is to identify the morphological and functional features of the lymphoid component of the tonsils in fetuses with intrauterine growth retardation (IUGR) in the late prenatal period. Material and Methods. The autopsy material of the study was tonsils from 10 full-term stillborn fetuses of average body weight (3.0-3.5 kg) (comparison group - hereinafter CG) and 11 stillborn fetuses weighing 2.1-2.5 kg (hereinafter - IUGR). The morphological material was stained by histological, histochemical methods, followed by morphometry on an Olympus BX-41 microscope with subsequent processing with Olympus DP-soft version 3.1 program. Immunohistochemical study was carried out using the direct Coons technique. The paraffin slices were treated with monoclonal antibodies (mAb) to Thy-1, CD3, CD4, CD5, CD8, CD19, CD22, HLA-Dr. The specimens were studied with the help of Carl Zeiss Axioskop 40 FL luminescent microscope and photographed with a CANON A520 digital camera. The light intensity was analyzed, determining the optical density of immunofluorescence of the immune cells using the original method. Results. Lymphoid follicles average diameter in IUGR group was 35.45±1.507 μm, which is significantly less than the value in CG group – 54.90±1.286 μm, (p ≤0.05). The relative volume of lymphoid tissue in the tonsils of the fetuses with IUGR was also significantly lower, amounting to 63.36±2.110% compared to 72.20±2.866% in CG group (p ≤0.05), the density of cellular elements in ×600 field of vision (IUGR group – 112.63±2.284, CG – 123.40±3.392; p ≤0.05). In IUGR group, the number of CD5 lymphocytes was significantly higher, amounting to 15.63±1.286 c.u. (in CG group, 12.30±1.159 c.u.) (p ≤0.05). The population of mature CD19 lymphocytes was significantly reduced in IUGR group (IUGR – 62.09±2.625 c.u., CG – 77.0±2.660 c.u.; p ≤0.05). The luminescence intensity of the areas, where CD3-mature T-lymphocytes were localized, was significantly reduced in IUGR group in comparison with the indicator of CG group (CG group – 55.70±1.828 c.u., IUGR – 51.72±1.190 c.u.; p ≤0.05). In IUGR group, CD4 population was significantly reduced – 33.72±2.101 c.u. vs. 45.80±1.813 c.u. in CG group (p ≤0.05), while CD8 population was enlarged (41.90±1.578 vs. 33.0±1.632 c.u. in CG group) (p ≤0.05). Conclusion. The results of the quantitative and qualitative assessment of the lymphoid component of the palatine tonsils of fetuses with IUGR revealed signs of hypoplasia and delayed maturation of both T- and B-lymphoid populations against the background of increased suppressor activity. Complete maturation may occur in the early stages of the postnatal development of the child, at the same time, an increase in the population of B-1 lymphocytes, as well as an increase in the suppressor activity of CD8, which has already been formed in utero, can later play the role of an important link in the morphogenesis of immunopathological reactions of various origins.

Key words: fetus, intrauterine growth retardation, palatine tonsils, immunomorphology.

Connection of the study with scientific programs, plans, topics
The research was conducted in accordance with KhNMU research project “Influence of maternal-fetal infection on the embryogenesis and fetogenesis of descendants”, state registration number 0115U000987.
foetuses with IUGR account for 28-45% among stillborns [5, 6]. One of the main morphogenetic links in IUGR development is chronic placental insufficiency due to somatic and infectious pathology of the mother, as well as complicated pregnancy [7, 8, 9]. The available literature data indicate that children with IUGR are at risk of perinatal morbidity and mortality [10]. The most common pathology of the perinatal period is postnatal asphyxia; later these children lag behind in neurophysic development, they have a tendency to hypoglycemia, which can manifest in the future as a tendency to diabetes mellitus and metabolic syndrome [11, 12, 13, 14]. Chronic diseases of the cardiovascular, respiratory, hepatobiliary systems as well as endocrinopathy are also more often observed in the adults born with IUGR [15, 16]. There is evidence that children born with IUGR are prone to infectious diseases in later ontogenesis due to the changes in their immune status programmed in the prenatal period, which is realized by disorders of both innate and acquired immunity [17, 18]. The data about the state of the immune system in foetuses and newborns with IUGR are limited and only the central organs of immunogenesis, such as the spleen and lymph nodes, have been investigated in depth [19, 20, 21, 22, 23], while the MALT system requires a more detailed study, taking into account the contemporary opportunities. The available information regarding MALT, including the tonsils, is scarce and is mainly based on experimental material [24, 25, 26, 27].

The aim of this work was to identify the morphological and functional features of the lymphoid component of the tonsils in foetuses with intrauterine growth retardation (IUGR) in the late prenatal period.

Material and Methods

The autopsy material of the study was tonsils from 10 full-term stillborn foetuses of average body weight (3.0–3.5 kg) (comparison group – hereinafter CG) and 11 stillborn foetuses weighing 2.1–2.5 kg (hereinafter – IUGR). The work was conducted in compliance with the basic provisions of the "Rules of ethical principles for conducting scientific medical research with the participation of humans", approved by Helsinki Declaration (1964-2013), ICH GCP (1996), EU Directive No. 609 (dated 24.11.1986), the order of the Ministry of Health of Ukraine No. 690 on 23.09.2009, No. 944 dated 14.12.2009, No. 616 dated 03.08.2012, approved by the Committee on Research Ethics of Kharkiv National Medical University. The tonsils were fixed in 10% neutral formalin and embedded in paraffin after alcohol dehydration. The paraffin sections 5-6 μm thick were stained with hematoxylin-eosin to identify the major structural components of the tonsils. The interstitial component was studied using staining according to Van Gieson and Mallory. PAS reaction was used. Microspecimens were investigated on Olympus BX-41 microscope with subsequent processing with Olympus DP-soft version 3.1 program, with which a morphometric study was carried out; the number of crypts and the number of lymphoid follicles were examined at magnification of ×80. Magnification ×200 was used to measure the diameter of the lymphoid follicle (30 measurements in 10 fields of vision). The area of the lymphoid tissue in the slice was measured with the calculation of the permeable volume. Immunohistochemical study was carried out using the direct Coons technique. The paraffin slices were treated with monoclonal antibodies (mAb) to Thy-1, CD3, CD4, CD5, CD8, CD19, CD22, HLA-Dr. The specimens were studied with the help of Carl Zeiss Axioskop 40 FL luminescent microscope and photographed with a CANON A520 digital camera. The light intensity was analyzed, determining the optical density of immunofluorescence of the immune cells using the original method [28]. Group mean values were compared using Student's t-test and Mann-Whitney U-test. Significance of differences between the values was taken at the level <0.05. Statistical calculations were performed using the Statistic Soft 6.0 program. The results are presented as M ± SD, where M is the arithmetic mean, and SD is the standard square deviation.

Results and Discussion

The tonsils in the foetuses of both groups were located in a triangular fossa between the palatoglossal arch frontally and the palatopharyngeal arch posteriorly. Their size did not exceed 3-4 mm, the shape was more often rounded, and the consistency was elastic.

Microscopically the free surface of the tonsils was lined with stratified squamous epithelium of unequal thickness. Subepithelial basement membranes were thin, PAS-positive, stained blue of varying intensity with Mallory staining. Basement membranes were not detected at the sites of contact with the epithelium of lymphoid follicles. As a rule, solitary cells with a large light nucleus and abundant cytoplasm, the so-called "valve cells", which are epithelial in nature, were located in this zone. It is in this zone, that antigens come into contact with the lymphoid tissue of the tonsils [29].

In all observations, narrow, branched crypts were noted in almost every field of vision (×80). The walls of the crypts were lined with stratified squamous epithelium. The crypt lumens were commonly free, sometimes containing a meager amount of desquamated cellular elements. Depending on the group of the fetus, the number of crypts on the tonsil section did not differ significantly, on an average it was 2.0 ± 0.666 in CG group and 1.72 ± 0.786 in IUGR group.

The parenchyma of the tonsils was represented by a reticular network containing lymphoid elements. The reticular fibers were stained blue by Mallory reaction, growth epithelioreticulocytes...
formed a finely looped network. In both groups, diffuse lymphoid tissue prevailed in the tonsils; lymphoid follicles were few (1-2 in the field of vision ×80) and small. Meanwhile, their average diameter in IUGR group was 35.45±1.507 μm, which is significantly less than the value in CG group – 54.90±1.286 μm, (p ≤0.05). Light centres were not detected in all follicles, which indicates the absence of antigenic stimulation [30]. The relative volume of lymphoid tissue in the tonsils of the foetuses with IUGR was also significantly lower, amounting to 63.36±2.110% compared to 72.20±2.898% in CG group (p ≤0.05). At the same time, the density of cellular elements in ×600 field of vision was also significantly lower, thus in IUGR group this indicator was 112.63±2.284, while in CG group it was 123.40±3.392 (p ≤0.05). In both observation groups, B lymphocytes expressing CD5, CD19, CD22 receptors dominated in the lymphoid component.

Interesting data were found at analysis of B-cell population composition. In IUGR group, the number of CD5 lymphocytes was significantly higher, amounting to 15.63±1.286 c.u. (in CG group, 12.30±1.159 c.u.) (p ≤0.05). CD5 receptors are known to express B1 lymphocytes, which are cells of bone-marrow origin. These cells are responsible for production of natural antibodies, rapid humoral immunity, and take part in the main immunoregulatory processes [31], moreover, they are prone to regulation of leukemic immunoregulatory processes [31], moreover, they are prone to regulation of leukemic immunoregulatory processes [31]. A similar pattern was also found in CD4 population, which makes up to 75% of all T-cells [34, 35]. A similar pattern was also found in the CG fetuses, in the tonsils of which the intensity of luminescence of CD4 population prevailed over that of CD8 population, while in the lymphoid tissue of the tonsils of fetuses with IUGR, the opposite pattern is noted (Fig. 1, 2). This is possibly due to the pathology of the mother, the analysis of which is being carried out at present and will be presented in the future communication along with the results of the study of the interstitial-vascular component.

**Conclusion**

1. The quantitative and qualitative assessment of the lymphoid component of the tonsils of fetuses with IUGR revealed significant features compared to those of the average-weight fetus. The lymphoid component of the tonsils of fetuses with IUGR has the signs of hypoplasia, which manifests by a significant reduction in the diameter of lymphoid
follies against a background of a significant reduction in the relative volume of lymphoid tissue. 2. Qualitative features of the lymphoid component were identified both among T-cell and B-cell populations. The number of CD5-B1-lymphocytes of bone-marrow origin was significantly increased, the population of mature CD19 lymphocytes was significantly reduced against a background of a trend towards an increase in the number of immature B-lymphocytes expressing HLA-Dr receptors. Among T-lymphocytes, the population of CD3-mature T-lymphocytes was significantly reduced, suppressor activity was increased, which is documented by predominance of CD8 lymphocytes over the CD4 population.

3. Our findings demonstrate, on the one hand, a delay in maturation of the main clones of immune cells of the tonsils at IUGR; complete maturation may occur in the early stages of the postnatal development of the child. At the same time, an increase in the population of B-1 lymphocytes, as well as an increase in the suppressor activity of CD8, which has already been formed in utero, can later play the role of an important link in the morphogenesis of immunopathological reactions of various origins.

Prospects for further research

Further morphological study of the interstitial and vascular components of the tonsils in IUGR will enable (by means of assessment of the parenchymal-stromal relationships) the identification of the main links in the morphogenesis of the above morphological features of the lymphoid component of the tonsils at IUGR, taking into account the pathology of the mother.

References

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Реферат
ПОРІВНЯЛЬНА ХАРАКТЕРИСТИКА ЛІМФОЇДНОГО КОМПОНЕНТА ПІДНЕБІННИХ МИГДАЛИКІВ ПЛОДІВ ІЗ ЗАТРИМКОЮ ВНУТРІШНЬОУТРОБНОГО РОЗВИТКУ І СЕРЕДНЬОЮ МАСОЮ ТІЛА В ПІЗНЬОМУ ПРЕНАТАЛЬНОМУ ПЕРІОДІ
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Ключові слова: плід, затримка внутрішньоутробного розвитку, піднебінні мигдалики, імуноморфологія.
Метою дослідження є виявлення морфофункціональних особливостей лімфоїдного компонента піднебінних мигдалин плодів із затримкою внутрішньоутробного розвитку у пізньому пренатальному періоді.
Матеріал та методи. Аутопсійний матеріал – піднебінні мигдалики від 10 доношених мертвонароджених плодів середньої маси тіла (3,0–3,5 кг) (група порівняння) і 11 мертвонароджених плодів з масою тіла 2,1–2,5 кг (основна група із затримкою внутрішньоутробного розвитку). Морфологічний матеріал забарвлювали гістологічними, гістохімічними методами з подальшою морфометрією на мікроскопі Olympus BX-41 з наступною обробкою програмою Olympus DP-soft version 3.1. Імуногістохімічні дослідження проводили за допомогою прямого методу Кунса. Парафінові зрізи обробляли монокліномічними антитілами (МКА) до Thy-1, CD3, CD4, CD5, CD8, CD19, CD22, HLA-Dr. Препарати вивчали з використанням люмінесцентного мікроскопа Carl Zeiss Axioskop 40 FL та фотографуванням цифровою камерою CANON A520 і аналізом інтенсивності світіння, визначаючи оптичну щільність імунофлюоресценції імунних клітин з власною методикою.
Результати. Середній діаметр лімфоїдних фолікулів мигдалин в основній групі становив 35,45±1,507 мкм, що достовірно менше показника в групі порівняння – 54,90±1,286 мкм, (р ≤0,05). Достовірно зменшений відносний об’єм лімфоїдної тканини в піднебінних мигдалинах плодів основної групи 63,36±2,110 % порівняно з 72,20±2,898 % у групи порівняння (р ≤0,05), щільність клітин у полі зору ×600, (основна група – 112,63±2,284 экз, група порівняння – 123,40±3,392; р ≤0,05).
В основній групі достовірно більшою виявилася кількість CD5 лімфоцитів, склавши 15,63±1,286 ум.од, (у групи порівняння – 12,30±1,159 ум.од) (р ≤0,05). Популяція зрілих CD19 лімфоцитів достовірно знижена в основній групі порівняно з 62,09±2,625 ум.од, група порівняння – 77,0±2,660 ум.од.; р ≤0,05). Інтенсивність світіння площин, де локалізувалися CD3-зрілі Т-лімфоцити, достовірно знижена в основній групі у порівнянні з показником групи порівняння (група порівняння – 55,70±1,828 ум.од., основна група – 51,72±1,190 ум.од.; р ≤0,05). Інтенсивність світіння плоскості, де локалізувалися CD22 з Rib-імунокомплексами, достовірно знижена в основній групі у порівнянні з показником групи порівняння (група порівняння – 41,90±1,578 ум.од проти 33,0±1,632 ум.од. у групи порівняння; р ≤0,05), збільшена популяція CD8 (41,90±1,578 ум.од проти 33,0±1,632 ум.од. у групи порівняння; р ≤0,05).

Висновки. Результати кількісної та якісної оцінки лімфоїдного компонента піднебінних мигдалин плодів із затримкою внутрішньоутробного розвитку, виявили ознаки гіпоплазії та затримкою дозрівання як Т-, так і B-лімфоїдних популяцій на фоні посilenня супресорної активності. І якщо повне дозрівання можливе на ранніх етапах постнатального розвитку дитини, то збільшення популяції В-1 лімфоцитів, а також підвищення супресорної активності CD8, що формується вже внутрішньоутробно, може надалі відіграти роль найважливішої ланки в морфогенезі імунопатологічних реакцій різного походження.