GEORGIAN MEDICAL NEWS

ISSN 1512-0112

No 12 (273) Декабрь 2017

ТБИЛИСИ - NEW YORK



ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

No 12 (273) 2017

Published in cooperation with and under the patronage of the Tbilisi State Medical University

Издается в сотрудничестве и под патронажем Тбилисского государственного медицинского университета

გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან თანამშრომლობითა და მისი პატრონაჟით



ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ ТБИЛИСИ - НЬЮ-ЙОРК

GEORGIAN MEDICAL NEWS

Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press; Georgian Academy of Medical Sciences; International Academy of Sciences, Education, Industry and Arts (USA).

Published since 1994. Distributed in NIS, EU and USA.

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ძუს კიბოს ბიოპტატები ბაზალურ-უჯრედოვანი ტიპის კარცინომის ჰისტოლოგიურ ვარიანტში.

გამოკვლეულია Cyclin E, CK5/17 и ER/PR რეცეპტორთა იმუნოექსპრესია.

დადგენილია, რომ აღნიშნულ ფაქტორებს

აქვთ დიფერენცირებული იმუნოექსპრესია, მაგრამ Cyclin E რეაქცია პირდაპირ აღმავალ კორელაციაში იმყოფება დაავადების კლინიკური სიმძიმის სტადიასა (III-IV) და არაკეთილსაიმედო პროგნოზთან.

VON WILLEBRAND FACTOR IMMUNOHISTOCHEMICAL STAINING QUANTITATIVE OPTICAL DENSITY PARAMETERS IN THE ENDOTHELIUM AND FIBRINOID OF THE PLACENTA DURING SECUNDINES INFLAMMATION AND CONCOMITANT IRON DEFICIENCY ANEMIA IN GRAVIDAS

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Von Willebrand factor is often used as an activation marker or marker of endothelial dysfunction [8,4]. In the recent years, other functions of von Willebrand factor have been identified, which suggests that this protein is involved in several other vascular processes [6]: angiogenesis and vascularization, proliferation of leiomyocytes. Shortly after von Willebrand factor was identified as a plasma protein, its ability to be adsorbed by collagen was reported as well. There are new suggestions about von Willebrand as a pro-inflammatory agent. An increased concentration of this protein was detected in plasma, particularly, in bacterial and viral infections and autoimmune diseases. It is suggested that the inflammation can be a general stimulus for the release of endothelial cells - the ULVWF (with ultrahigh von Willebrand factor), which can lead to a deficiency of ADAMTS13-metaloproteinase, which, in turn, is able to break up and formulate its multimers of normal and low molecular weight. As a result, ULVWF multimers can be stored in endothelial cells of blood vessels and blood plasma for a long time to cause adhesion and aggregation of platelets, which can lead to thrombosis. This confirms the connection between the inflammation and thrombosis. [2].

The quantitative characteristics concerning the optical density of von Willebrand factor immunohistochemical staining in the placenta are to be found only in several studies [3-5, 9], but the aspect of inflammation and iron deficiency anemia in pregnant women has not been addressed in these studies.

The aim of the study was to establish optical density quantitative parameters of von Willebrand factor immunohistochemical staining in the endothelium and fibrinoid of placenta during acute and chronic inflammation of the secundines combined with iron deficiency anemia in pregnant women.

Materials and methods. The total number of 198 placentas was examined, including those studied during

physiological pregnancy on the background of iron deficiency anemia in gravidas without the secundines inflammation (for comparison purposes).

The numbers observed in specific research groups are presented in Tables 1 and 2.

The material was kept in a buffered neutral 10% formalin solution for 20-22 hours, followed by dehydrating in the ascending battery of alcohols and placing in paraffin at 56*C. The immunohistochemical techniques were performed on sections made from the paraffin blocks (after deparaffinization) using the von Willebland visualization of primary antibodies by a polymeric system (DAKO) with a diaminobenzidine dye. Digital copies of the image were obtained using the microscope Delta Optical Evolution 100 (Planar Lenses) and the digital camera Olympus SP-550UZ. The method of computer microdensitometry was implemented in the medium of computer program ImageJ (1.48, W. Rasband, National Institutes of Health, USA) [10]. The optical density of the histochemical staining was measured in relative units (in the range from 0 to 1, based on the logarithmic transformations of the brightness index in gradations from 0 to 255).

The arithmetic mean and its error (for optical density) were calculated using the computer program PAST 3.16 (free license, O. Hammer, 2017) [7]. Differences in the average tendencies were determined with the help of a two-sided odd t-test with a preliminary check on the normality of distribution in statistical samples. Statistically significant differences were considered at p \leq 0.05.

Results and their discussion. The inflammation was diagnosed based on the histological sections having been stained with hematoxylin and eosin. Polymorphonuclear leukocytes with the admixture of individual lymphocytes prevailed in acute forms of inflammation in the nidus of infection. In chronic forms of inflammation lymphocytes were predominantly determined in the focus of research.

In both forms of inflammation, altered changes in local cells and interstitial edema were observed.

During the course of conducting immunohistochemical studies it was found that the specific von Willebrand factor immunohistochemical staining was noticed in several structures of the placenta: in endothelial cells of blood vessels, in the fibrinoid at different localizations, and sometimes on the surface of the trophoblasts of the placental chorionic villi. Our article presents the results of quantitative parameters of the optical density of the immunohistochemical staining for the von Willebrand factor of the endothelial cell cytoplasm and the fibrinoid of the chorionic and basal plates in accordance with the localization of the inflammatory process.

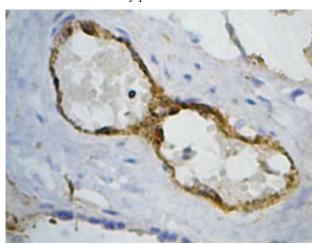


Fig.1. Von Willebrand factor in the endothelium of the blood vessel of the chorionic plate of the placenta. Observation of the physiological pregnancy. Immunohistochemical technique using primary antibodies against von Willebrand factor. Ob.40°.Oc.10°

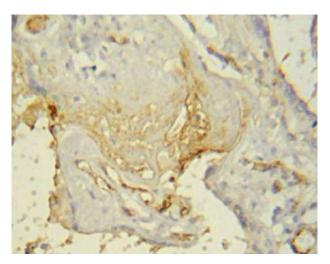


Fig.3. Von Willebrand factor in the fibrinoid of the chorionic plate of the placenta. Observation of the physiological pregnancy. Immunohistochemical technique using primary antibodies against von Willebrand factor. Ob. $40^{\rm x}$. Oc. $10^{\rm x}$

During the physiological pregnancy the intensity of the staining for the von Willebrand factor was more pronounced in the endothelium of the blood vessels (Fig. 1, 2), in comparison to the fibrinoid (Fig. 3, 4), with the degree of perforation varied considerably among the endothelial cells of the blood vessel. Although, it was characterized by the same feature. Small groups of various shaped circular cells were found in the histological sections of placenta preparations with the signs of chronic inflammation. These cells were also intensively stained for the von Willebrand factor. This phenomenon can probably be interpreted as the process of neoplasm of blood vessels, i.e. these facts reflect the processes of angiogenesis.

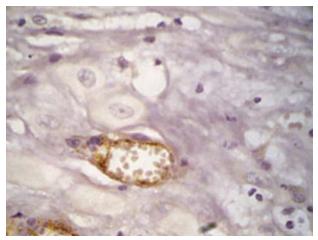


Fig.2. Von Willebrand factor in the endothelium of the blood vessel of the basal plate of the placenta. Observation of the physiological pregnancy. Immunohistochemical technique using primary antibodies against von Willebrand factor. Ob.40°.Oc.10°

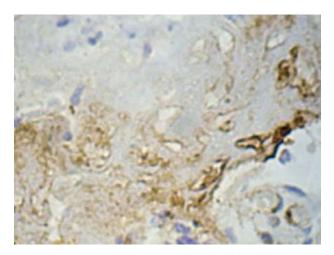


Fig.4. Von Willebrand factor in the fibrinoid of the basal plate of the placenta. Observation of the physiological pregnancy. Immunohistochemical technique using primary antibodies against von Willebrand factor. Ob.40°.Oc.10°

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Table 1. Optical density of the immunohistochemical staining in the fibrinoid and endotheliocytes of the blood vessels of the placenta (immunohistochemical technique using primary antibodies against von Willebrand factor) during physiological pregnancy and iron deficiency anemia in pregnant women (M±m)

	Research groups	
Structures	Observation of physiological pregnancy (n=20)	Observation of non-inflammatory iron deficiency anemia in pregnant women (n=21)
Endotheliocytes of the chorionic plate	0,228±0,0025	0,322±0,0021 (P<0,001)
Fibrinoid of the chorionic plate	0,124±0,0021	0,149±0,0020 (P<0,001)
Endotheliocytes of the basal plate	0,374±0,0022	0,385±0,0028 (P=0,003)
Fibrinoid of the basal plate	0,142±0,0019	0,168±0,0022 (P<0,001)

Table 2. Optical density of the immunohistochemical staining in the endotheliocytes of the blood vessels of the placenta (immunohistochemical technique using primary antibodies against von Willebrand factor) in combination with different forms of the secundines inflammation and iron deficiency anemia in pregnant women (M±m)

Research groups	Observation of the secundines inflammation in pregnancy without anemia	Observation of the secundines inflam- mation in iron deficiency anemia of pregnant women
Acute chorioamnionitis (endothelium of the chorionic plate was studied)	0,323±0,0024 (n=23)	0,386±0,0025 (n=21) P<0,001
Chronic chorioamnionitis (endothelium of the chorionic plate was studied)	0,328±0,0025 (n=20)	0,385±0,0027 (n=21) P<0,001
Acute basal deciduitis (endothelium of the basal plate was studied)	0,396±0,0027 (n=16)	0,408±0,0029 (n=15) P=0,005
Chronic basal deciduitis (endothelium of the basal plate was studied)	0,398±0,0029 (n=21)	0,416±0,0032 (n=20) P=0,005

Table 3.Optical density of the immunohistochemical staining in the fibrinoid of the placenta (immunohistochemical technique using primary antibodies against von Willebrand factor) in combination with different forms of the secundines inflammation and iron deficiency anemia in pregnant women (M±m)

Research groups	Observation of the secundines in- flammation in pregnancy without anemia	Observation of the secundines inflammation in iron deficiency anemia of pregnant women
Acute chorioamnionitis (fibrinoid of the chorionic plate was studied)	0,126±0,0029 (n=23)	0,152±0,0028 (n=21) P<0,001
Chronic chorioamnionitis (fibrinoid of the chorionic plate was studied)	0,158±0,0030 (n=20)	0,171±0,0036 (n=21) P=0,009
Acute basal deciduitis (fibrinoid of the basal plate was studied)	0,146±0,0024 (n=16)	0,170±0,0029 P=0,001 (n=15)
Chronic basal deciduitis (fibrinoid of the basal plate was studied)	0,170±0,0033 (n=21)	0,181±0,0034 (n=20) P=0,026

As to the fibrinoid, of both chorionic and basal plates, it should be mentioned that the von Willebrand factor in it was visualized in the form of thread-like chaotic oriented structures.

For the purpose of comparison, the optical density quantitative analysis of the von Willebrand factor immunohistochemical staining was conducted in the endothelium and fibrinoid of the placenta during physiological pregnancy and iron deficiency anemia in gravidas. Average data on the processes, which concern the observations of physiological pregnancy and iron deficiency anemia are given in Table 1.

It is obvious from the previously mentioned data that the intensity of the optical density of staining in the endothelial cells and fibrinoid in physiological pregnancy in different placental structures significantly differs. The von Willebrand factor is higher in endothelial cells than in fibrinoid, which confirms the findings of a visual assessment of the intensity of histological sections perforation, and the staining is most pronounced in the basal plate of the placenta.

Furthermore, iron deficiency anemia in gravidas (IDAG) causes an increase in the optical density of staining in all the studies, with the maximum indices in the basal plate of the placenta, both in the endothelial cells and in the fibrinoid.

These indicators are important in terms of estimating the optical density of the immunohistochemical staining as the key indicator of von Willebrand factor level.

According to the data given in Table 2, it was found that in all the forms of inflammation of the secundines and the structures under study, the optical density of the von Willebrand immunohistochemical staining in the endothelium of the blood vessels significantly increases in comparison with the physiological pregnancy. However, in relation to non-inflammatory IDAG, there are no statistically significant mean differences in the average trends between observations. The inflammation on the background of iron deficiency anemia in gravidas contributes to a rapid increase in the indices, with the highest data in the endothelial cells of the basal plate in chronic basal deciduitis.

At the same time, it was marked that, as an average tendency, anemia is accompanied by statistically higher significant indicators in acute and chronic forms of chorioamnionitis than in basal deciduitis.

According to the data in Table 3, only in chronic forms of chorioamnionitis and basal deciduitis, the optical density of staining in the fibrinoid of the chorionic and basal plate of the placenta, is higher than in physiological pregnancy. And in comparison with IDAG in absence of the inflammation, the intensity of staining increases only in the fibrinoid of the chorionic plate in chronic chorioamnionitis, while in acute forms, the indices are significantly lower than in the comparison group. It was found, at the same time, that the quantitative parameters of the von Willebrand factor staining optical density in all the inflammatory forms combined with IDAG, significantly increase

in the corresponding structures of the placenta, in comparison with physiological pregnancy and inflammation of the placenta, with the maximum numbers reported during acute chorioamnionitis and basal deciduitis. It should be emphasized that only chronic inflammatory processes in combination with IDAG cause a change in the indices compared with IDAG without an inflammation.

Conclusions. 1. The intensity of the von Willebrand factor staining optical density in the endothelium of the blood vessels is more pronounced in comparison with the fibrinoid. This pattern is observed both in cases of physiological pregnancy and the secundines inflammation.

- 2. The von Willebrand factor immunohistochemical staining optical density is significantly increased in the endothelium of blood vessels in all forms of the secundines inflammation, in comparison with physiological pregnancy. At the same time, iron deficiency anemia of pregnant women is accompanied with maximum levels of optical density in the endothelium, whereas in chronic forms of inflammation, the average indices are higher than those for acute forms of inflammation.
- 3. In the fibrinoid of the chorionic or basal plate, the staining optical density of von Willebrand factor does not change in acute forms of the secundines inflammation, but increases with chronic forms. In this case, iron deficiency anemia in pregnant women is accompanied by maximum levels of the staining optical density in the fibrinoid, and in chronic forms of inflammation, the average indices are higher than those with the acute forms of inflammation.

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SUMMARY

VON WILLEBRAND FACTOR IMMUNOHISTO-CHEMICAL STAINING QUANTITATIVE OPTI-CAL DENSITY PARAMETERS IN THE ENDO-THELIUM AND FIBRINOID OF THE PLACENTA DURING SECUNDINES INFLAMMATION AND CONCOMITANT IRON DEFICIENCY ANEMIA IN GRAVIDAS

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The aim of the research was to set the optical density quantitative parameters of the von Willebrand factor immunohistochemical staining (vWF) in the endothelium and fibrinoid of the placenta during the secundines inflammation concomitant with iron deficiency anemia in gravidas.

The total number of 198 placentas was studied. The immunohistochemical technique was performed using the visualization of the primary antibodies to vWF with a diaminobenzidine dye polymer system. The optical density of the histochemical staining was measured by means of computer microdensitometry after the digital copies of the images had been obtained.

All the cases of the secundines inflammation and the structures under study were found to have a significant increase in the optical density of the vWF immunohistochemical staining in the endothelium of the blood vessels as compared to the physiological pregnancy. Iron deficiency anemia in gravidas (IDAG) contributes to an increase in the indices of the inflammation, the highest data pertaining to the endothelial cells of the placental basal plate in chronic basal deciduitis.

The optical density of the staining in the fibrinoid of the chorionic and basal plates during chronic forms of chorioamnionitis and basal deciduitis is higher than the optical density inherent in physiological pregnancy. The intensity of staining increases in presence of all the forms of inflammation on the background of IDAG in comparison with physiological pregnancy with placenta inflammation. Compared with IDAG in absence of the inflammatory processes, only chronic inflammatory processes reveal a change in indices.

Consequently, the optical density of the staining significantly increases in the endothelium of blood vessels in all forms of the secundines inflammation, in comparison with the physiological pregnancy, whereas in fibrinoid the same process is reported only in chronic course. In this case, IDAG is accompanied by maximum levels of optical density in the endothelium and fibrinoid, whereas in chronic, the average indices are higher than those in acute forms.

Keywords: von Willebrand factor, inflammation of the placenta, iron-deficiency anemia in gravidas.

РЕЗЮМЕ

КОЛИЧЕСТВЕННЫЕ ПАРАМЕТРЫ ОПТИЧЕСКОЙ ПЛОТНОСТИ ИММУНОГИСТО-ХИМИЧЕСКОЙ ОКРАСКИ НА ФАКТОР von WILLEBRAND В ЭНДОТЕЛИИ И ФИБРИНОИ-ДЕ ПЛАЦЕНТЫ ПРИ СОЧЕТАННЫХ ВОСПА-ЛЕНИИ ПОСЛЕДА И ЖЕЛЕЗОДЕФИЦИТНОЙ АНЕМИИ У БЕРЕМЕННЫХ

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Целью исследования явилось установление количественных параметров оптической плотности иммуногистохимического окрашивания на фактор фон Виллебранда в эндотелии и фибриноиде плаценты при сочетанном воспалении последа и железодефицитной анемии беременных.

Изучено 198 плацент. Иммуногистохимическую оценку проводили с использованием первичных антител на фактор фон Виллебранда (vWF) с визуализацией полимерной системой красителя диаминобензидина. Оптическая плотность гистохимического окрашивания измерялась с помощью компьютерной микросенситометрии после получения цифровых копий изображения.

Обнаружено, что в сравнении с физиологической беременностью при наличии воспаления последа и исследуемых структур значительно увеличена оптическая плотность иммуногистохимического окрашивания на vWF в эндотелии кровеносных сосудов. Железодефицитная анемия беременных (IDAG) способствует повышению показателей воспаления.

Оптическая плотность окрашивания в фибриноиде хорионической и базальной пластинки выше, чем оптическая плотность при физиологической беременности только при наличии хронических форм хориоамнионита и воспаления базальной мембраны. По сравнению с физиологической беременностью и воспалением плаценты, повышенная интенсивность окрашивания отмечается при всех формах воспаления, сочетанного с IDAG. Сравнительный анализ показал, что изменение показателей отмечается только при хронических воспалительных процессах и отсутствует при наличии IDAG без воспаления.

Следовательно, по сравнению с физиологической беременностью, при всех формах воспаления последа оптическая плотность окрашивания в эндотелии кровеносных сосудов значительно возрастает, тогда как в фибриноидах такой же процесс прослеживается только при хроническом течении. В этом случае IDAG сопровождается максимальными уровнями оптической плотности в эндотелии и фибриноиде, тогда как при хроническом воспалении средние показатели выше, чем при острой форме.

რეზიუმე

იმუნოჰისტოქიმიური შეფერადების ოპტიკური სიმკვრივის რაოდენობრივი პარამეტრები ენდოთელიუმისა და პლაცენტის ფიბრინოიდის Willebrand-ის ფაქტორზე

ვ. ილიკა, ნ. დავიდენკო

უმაღლესი სახელმწიფო საგანმანთლებლო დაწესებულება "ბუკოვინის სახელმწიფო სამედიცინო უნივერსიტეტი", ჩერნოვცი, უკრაინა

კვლევის მიზანს შეადგენდა იმუნოპისტოქიმიური შეფერადების ოპტიკური სიმკვრივის რაოდენობრივი პარამეტრების დადგენა Willebrand-ის ფაქტორზე პლაცენტის გარკვეული სტრუქტურების ანთებისა და ორსულების რკინადეფიციტური ანემიის (IDAG) თანხვედრის დროს.

გამოკვლეულია 198 პლაცენტა. იმუნოპისტოქიმიური მეთოდიკა სრულდებოდა პარაფინურ
ანათლებზე Willebrand-ის ფაქტორის მიმართ
პირველადი ანტისხეულების გამოყენებით და
მათი ვიზუალიზაციით დიამინობენზიდინიანი
პოლიმერული სისტემით. კომპიუტერული მიკროდენსიტომეტრიის მეთოდით გამოსახულების
ციფრული ასლების მიღების შემდგომ იზომებოდა ჰისტოქიმიური შეფერადების ოპტიკური
სიმკვრივე.

დადგენილია, რომ შესწავლილ სტრუქტურებში ანთების ყველა ფორმის დროს,ფიზიოლოგიურ ორსულობასთან შედარებით, საშუალოდ, იმუნოჰისტოქიმიური შეფერადების ოპტიკური სიმკვრივე Willebrand-ის ფაქტორზე მნიშვნელოვნად მატულობს. IDAG ანთების დროს ხელს უწყობს ამ მაჩვენებლის ზრდას, ამასთან, ყველაზე მაღალი მაჩვენებლები დაფიქსირდა პლაცენტის ბაზალური ფირფიტის ენდოთელიოციტებზე ქრონიკული ბაზალური დეციდუიტის დროს.

ქორიონული და ბაზალური ფირფიტის ფიბრინოიდში ქორიონამნიონიტის ქრონიკული ფორმების და ბაზალური დეციდუიტის დროს შეფერადების სიმკვრივე მეტია, ვიდრე ფიზიოლოგიური ორსულობისას. IDAG და ანთების ყველა ფორმის დროს, ფიზიოლოგიურ ორსულობასა და პლაცენტის ანთებასთან შედარებით, შეფერადების სიმკვრივე იზრდება.

ამრიგად, სისხლძარღვების ენდოთელიუმში ანთების ყველა ფორმის დროს, ფიზიოლოგიურ ორსულობასთან შედარებით, შეფერადების ოპტიკური სიმკვრივე მატულობს, ფიბრინოიდში კი — მხოლოდ ქრონიკული მიმდინარეობის პირობებში. ამასთან, IDAG-ს თან ახლავს ოპტიკური სიმკვრივის მაქსიმალური მაჩვენებლები ენდოთელიუმსა და ფიბრინოიდში, ხოლო ქრონიკული ანთების დროს საშუალო მაჩვენებლები მწვავე ფორმების მაჩვენებლებზე მაღალია.

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