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ORIGINAL ARTICLE

THE FEATURES OF LIMITED PROTEOLYSIS IN PLACENTAL FIBRINOID IN COMBINATION WITH INFLAMMATION AND IRON DEFICIENCY ANEMIA OF PREGNANT WOMEN

DOI: 10.36740/WLek202305121

Vitalii V. Ilika, Oleksandra V. Garvasiuk, Oleksandra I. Dogolich, Batih V. Iryna

BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

The aim: To establish the features of limited proteolysis in fibrinoid of the chorionic and basal plates of the placenta in acute and chronic chorioamnionitis, as well as basal deciduitis on the background of iron deficiency anemia in pregnant women.

Materials and methods: The histochemical procedure was performed using the ninhydrin-Schiff response to free amino groups of proteins by the method of A. Yasuma and T. Ichikawa, and Bonheg bromophenol blue.

Results: With iron deficiency anemia of pregnant women, the relative units of optical density in the chorionic plate were 0.312 ± 0.0026 , and with basal one – 0.310 ± 0.0024 (with indicators of physiological pregnancy 0.285 ± 0.0024 and 0.289 ± 0.0021). In the observations of acute chorioamnionitis, the quantitative indicators were 0.311 ± 0.0024 , chronic one – 0.311 ± 0.0024 , and with inflammation on the background of anemia of pregnant women – 0.315 ± 0.0031 and 0.339 ± 0.0036 , respectively. With acute basal deciduitis – 0.316 ± 0.0027 , chronic one – 0.326 ± 0.0034 , and with inflammation of the basal plate of the placenta on the background of anemia of pregnant women – 0.320 ± 0.0031 and 0.341 ± 0.0038 , respectively.

Conclusions: With anemia of pregnant women, the processes of limited proteolysis are intensified in accordance with the indicators of optical density of histochemical staining in the fibrinoid of the chorionic and basal plates of the placenta compared with physiological pregnancy. In case of acute and chronic forms of chorioamnionitis and basal deciduitis, quantitative indicators of optic density of histochemical staining increase compared with physiological pregnancy. Comorbid anemia of pregnant women activates the processes of limited proteolysis only in the chronic form of chorioamnionitis and basal deciduitis.

KEY WORDS: placental insufficiency, chorioamnionitis, basal deciduitis, protein amino groups, free radical processes

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INTRODUCTION

Many scientific papers have been devoted to the problem of inflammation of the manure and iron deficiency anemia (IDA) of pregnant women due to the high frequency of these conditions. However, it is important to investigate their interaction, in order to expand and supplement the information base of the pathomorphology of placental insufficiency, which is a common morphological manifestation for these conditions.

After analyzing domestic and foreign literature, we noticed that the morphology of chorioamnionitis (ChA) and basal deciduitis (DB) on the background of iron deficiency anemia in pregnancy is not given much attention, the study of which is essential to establish morpho-functional features and optimize diagnosis and development of complications and diseases of newborn. You can try to predict pathological changes in the placenta in advance by finding data on pathophysiological and morphological changes in the interaction of these conditions in the human body in many other

inflammatory processes in the absence of pregnancy, however, it is not necessary to unequivocally assert their regularity in the placenta in case of IDA during pregnancy, because the placenta, with its inherent features (absence of lymphatic vessels and nerve endings, immunological signs [1–3], circulatory properties in the fetal part of the placenta (venous blood flows in arteries, and arterial blood flows in veins) [1], can dramatically change the morphology of manure inflammation. Uncertainty on the above issues complicates the pathological diagnosis of the placenta and needs to be resolved.

It is known that many pathological processes, as a rule, increase the level of oxygen free radicals, which oxidize the amino groups of proteins, change the properties of these macromolecules [4]. Intensification of oxidative modification of proteins may be accompanied by enhanced processes of limited proteolysis [5], which is associated with fundamental life processes: intracellular protein breakdown and regulation of

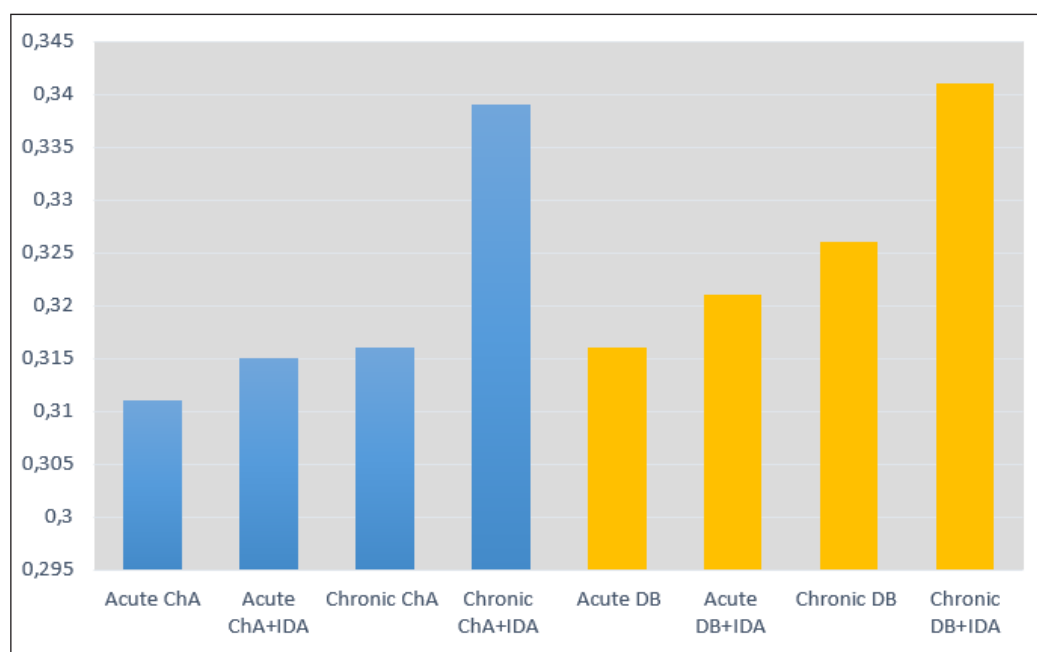


Fig. 1. Comparison of optical density of histochemical staining for free amino groups of proteins in the fibrinoid of the chorionic and basal plates of the placenta in inflammation on the background of IDA during pregnancy

their elimination, nutrition, morphogenesis, protective reactions, and adaptive rearrangements of metabolism [6]. In this aspect, our previous publications highlight our findings on the study of nitroperoxides [7] and determine the degree of oxidative modification of proteins in inflammation of manure in combination with iron deficiency anemia in pregnancy [8]. However, the features of limited proteolysis in the combination of these conditions have not yet been elucidated and are promising in order to fully understand the histochemical properties of proteins in placental structures.

THE AIM

To establish the features of limited proteolysis in fibrinoid of the chorionic and basal plates of the placenta with iron deficiency anemia of pregnant women, in acute and chronic chorioamnionitis, as well as basal deciduitis on the background of iron deficiency anemia in pregnant women by means of histochemical method in combination with computer microdensitometry.

MATERIALS AND METHODS

198 placentas from parturients at 37 – 40 weeks of gestation were studied. Including, for comparison, the placenta during physiological pregnancy and the observation of iron deficiency anemia of pregnant women without inflammation of the manure. The number of observations in specific study groups is given in the tables (Table I and Table II). The study of fibrinoid in chorioamnionitis was studied in the chorionic, and in basal deciduitis - in the decidual plate of the placenta.

To achieve the goal and solve the tasks set in this study, the following were carried out: histochemical (histochemical technique using ninhydrinoschiffian reaction to free amino groups of proteins by the method of A. Yasuma and T. Ichikava, histochemical method for total protein with bromophenol blue according to Bonheg, morphometric (in ImageJ computer program environment) and statistical research methods (an odd Student's test and Shapiro-Wilk test).

Pieces of placenta were fixed for 24 to 48 hours in neutral Lilly-buffered 10% formalin solution, followed by ethanol dehydration and paraffin pouring according to standard procedures. On serial histological sections of 5 µm thick there were performed 2 methods: 1) histochemical technique using ninhydrinoschiffian reaction to free amino groups of proteins by the method of A. Yasuma and T. Ichikava, which allows to assess the degree of limited proteolysis, resulting in "opening" hidden amino groups of proteins; 2) histochemical method for total protein with bromophenol blue according to Bonheg.

Digital copies of the image were obtained using a Delta Optical Evolution 100 microscope (planachromatic lenses) and an Olympus SP-550UZ digital camera. The obtained images were analyzed in the computer program ImageJ (1.48, W. Rasband, National Institutes of Health, USA) [9].

Quantitative measure of total protein and limited proteolysis was the value of relative optical density (relative units of optical density) in units of optical density (from 0 - no color, absolute transparency, to 1 - maximum color, absolute opacity, based on logarithmic transformations) in gradations from "0" to "255".

Table I. Optical color density (relative to optical density) in the fibrinoid of the basal and chorionic plates in physiological pregnancy and iron deficiency anemia of pregnant women ($M \pm m$)

Structures	Study Group	
	Observation of physiological pregnancy (n=20)	Observation of iron deficiency anemia of pregnant women without inflammation of the manure (n=21)
Fibrinoid of chorionic plate	0.285±0.0024	0.312±0.0026 p<0.001
Fibrinoid of basal plate	0.289±0.0021	0.310±0.0024 p<0.001

Note: p – the probability of the difference between the two means between physiological pregnancy and the study group

Table II. Optical color density (relative to optical density) in fibrinoid of the chorionic and basal plates of the placenta with a combination of different forms of inflammation of manure and iron deficiency anemia of pregnant women ($M \pm m$)

Study Group	Observation of inflammation of the manure	Observation of inflammation of the manure
Chorioamnionitis acute (fibrinoid of the chorionic plate is studied)	0.311±0.0024 p ₁ <0.001 (n=23)	0.315±0.0031 p ₂ >0.05 (n=21)
Chorioamnionitis chronic (fibrinoid of the chorionic plate is studied)	0.316±0.0024 p ₁ <0.001 (n=20)	0.339±0.0036 p ₂ <0.001 (n=21)
Basal decidualitis acute (fibrinoid of the basal plate is studied)	0.316±0.0027 p ₁ <0.001 (n=16)	0.320±0.0031 p ₂ >0.05 (n=15)
Basal decidualitis chronic (fibrinoid of the basal plate is studied)	0.326±0.0034 p ₁ <0.001 (n=21)	0.341±0.0038 p ₂ <0.001 (n=20)

Note: p₁ - the probability of the difference between the two means between physiological pregnancy and the study group; p₂ - the probability of the difference between the two means between inflammation of the studied placental plate and inflammation in combination with IDA of pregnant women

Arithmetic mean and its error were calculated, discrepancies in mean tendencies were checked using an odd Student's test after a positive test of the sample for normality of distribution in it according to Shapiro-Wilk test (computer program PAST 3.14, free license, O. Hammer, 2016) [10]. The critical value of the level of significance (p) was accepted at $p \leq 0.05$, which could indicate the influence of iron-deficiency anemia of pregnant women on the morphology of placental inflammation. If a value of $p > 0.05$ was obtained, the difference between the values was considered unreliable.

All studies were performed in compliance with the Council of Europe Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (March 18, 1986) "Ethical Principles for Medical Research Involving Human Subjects", approved by the Declaration of Helsinki (1964 - 2013), ICH GCP (1996), EEC Directives #609 (dated 24.11.1986), Orders of the Ministry of Health of Ukraine #690 dated 23.09.2009, #944 dated 14.12.2009, #616 dated 03.08.2012. The Commission on Bioethics of the Bukovinian State Medical University of Ukraine found no violations (Minutes #4 of December 19, 2019).

All the procedures and experiments of this study respect the ethical standards of the Helsinki Declaration of 1975, as revised in 2008 (5), as well as the national law. Informed consent was obtained from all the patients included in the study.

RESULTS

Specific histochemical staining in fibrinoid, endothelial cells and placental decidual cells was identified on the obtained histological preparations using the ninhydrin-schiffian reaction to free amino groups of proteins by the method of A. Yasuma and T. Ichikawa. Previously, we have conducted research using this technique in endothelial cells of the chorionic and basal plates of the placenta [11]. This part of the paper is devoted to the quantitative assessment of the degree of limited proteolysis by determining the optical density of histochemical staining in fibrinoid in chorioamnionitis and basal decidualitis on the background of IDA in pregnancy.

Thus, according to the quantitative indicators of the stoichiometric study using the ninhydrin-Schiff reaction

Table III. Optical density of staining for total protein according to Bonheg in fibrinoid (relative units of optical density) of basal and chorionic plates of the placenta during physiological pregnancy and iron deficiency anemia of pregnant women ($M \pm m$)

Structures	Study Group	
	Observation of physiological pregnancy (n=20)	Observation of iron deficiency anemia of pregnant women without inflammation of the manure (n=21)
Fibrinoid of chorionic plate	0.412 $\pm$ 0.0114	0.421 $\pm$ 0.0102 $p > 0.05$
Fibrinoid of basal plate	0.408 $\pm$ 0.0104	0.416 $\pm$ 0.0110 $p > 0.05$

Note: p – the probability of the difference between the two means between physiological pregnancy and the study group

Table IV. Optical color density for total protein according to Bonheg (relative units of optical density) in placental fibrinoid with a combination of different forms of inflammation of manure and iron deficiency anemia of pregnant women ($M \pm m$)

Study Group	Observation of inflammation of the manure	Observation of inflammation of the manure
Chorioamnionitis acute (fibrinoid of the chorionic plate is studied)	0.410 $\pm$ 0.0110 $p_1 > 0.05$ (n=23)	0.412 $\pm$ 0.0112 $p_2 > 0.05$ (n=21)
Chorioamnionitis chronic (fibrinoid of the chorionic plate is studied)	0.414 $\pm$ 0.0109 $p_1 > 0.05$ (n=20)	0.417 $\pm$ 0.0114 $p_2 > 0.05$ (n=21)
Basal deciduitis acute (fibrinoid of the basal plate is studied)	0.407 $\pm$ 0.0106 $p_1 > 0.05$ (n=16)	0.405 $\pm$ 0.0104 $p_2 > 0.05$ (n=15)
Basal deciduitis chronic (fibrinoid of the basal plate is studied)	0.404 $\pm$ 0.0104 $p_1 > 0.05$ (n=21)	0.401 $\pm$ 0.0102 $p_2 > 0.05$ (n=20)

Note: p_1 – the probability of the difference between the two means between physiological pregnancy and the study group; p_2 – the probability of the difference between the two means between the study group and inflammation without anemia

to free amino groups of proteins by the method of A. Yasuma and T. Ichikawa, which are given in the table (Table I and Table II), we can see that in case of IDA in pregnancy relative units of optical density increase on average with the same intensity in both the chorionic and basal plates of the placenta (0.312 $\pm$ 0.0026, $p < 0.001$ and 0.310 $\pm$ 0.0024, $p < 0.001$, respectively).

When studying the processes of limited proteolysis in the observations of acute and chronic chorioamnionitis (0.311 $\pm$ 0.0024 and 0.316 $\pm$ 0.0024), the obtained indicators showed an intensive increase in quantitative parameters relative to physiological pregnancy (0.285 $\pm$ 0.0024), where $p < 0.001$. Only in chronic inflammation on the background of IDA in pregnancy the processes of limited proteolysis in the fibrinoid of the chorionic plate of the placenta (0.339 $\pm$ 0.0036, $p < 0.001$) were intensified.

Regarding the fibrinoid of the placental basal plate, these tables convincingly show that in both acute (0.316 $\pm$ 0.0027) and chronic (0.326 $\pm$ 0.0034) basal deciduitis the processes of limited proteolysis were higher compared to observations of physiological pregnancy ($p < 0.001$), with the highest indicators of chronic inflammation. Iron deficiency anemia of pregnant

women affects the intensification of processes only in chronic inflammation of the basal plate of the placenta (0.341 $\pm$ 0.0038, $p < 0.001$).

Visualization of the comparative characteristics between acute and chronic inflammation of both the chorionic and basal plates of the placenta can be performed using the figure (Fig. 1). When comparing, we can see a clear pattern of growth of the processes of limited proteolysis both in acute and chronic chorioamnionitis and basal deciduitis concerning the placentas of physiological pregnancy in the corresponding plates of the placenta. However, the obtained quantitative indicators of the relative units of optical density of histochemical staining in the observations of basal deciduitis are higher than in the observations of chorioamnionitis. Iron-deficiency anemia of pregnant women has the greatest effect on the intensification of the processes of limited proteolysis in chronic inflammation, with the highest indicators in chronic basal deciduitis.

Iron-deficiency anemia of pregnant women has the greatest effect on the intensification of the processes of limited proteolysis in chronic inflammation, with the highest indicators in chronic basal deciduitis.

DISCUSSION

Our data on the increase on average with the same intensity of relative units of optical density of histochemical staining for free amino groups of proteins in both the chorionic and basal plates of the placenta in anemia of pregnant women do not contradict the results of other researchers, which demonstrate that the processes of limited proteolysis intensify under the conditions of IDA of pregnant women. In particular, the authors found an increase in the processes of limited proteolysis in the trophoblast cytoplasm of the chorionic villi of the placenta in combination with premature maturation of the chorionic tree and iron deficiency anemia [12]. According to a team of other authors, according to a histochemical study of iron-deficiency anemia of pregnant women in fibrinoid with type II and type IV calcium deposits (fine-grained deposits), both in the zone of the chorionic tree and in the basal plate of the placenta, a sharp increase in the processes of limited proteolysis was established in comparison with the observations without anemia [13].

The expected results were obtained in acute and chronic chorioamnionitis, as well as basal deciduitis, which indicate the intensification of limited proteolysis. Thus, the most limited proteolysis, according to the optical density of a specific color, increases with inflammation of the basal plate of the placenta compared with the indicators of physiological pregnancy and IDA of pregnant women without inflammation. These quantitative indicators are the highest in the chronic form. Slightly less, but with a high statistical probability, limited proteolysis is enhanced by inflammation of the chorionic plate. It should be noted that inflammation of the manure in combination with IDA was characterized by higher average figures than without anemia, only in chronic inflammation of both the chorionic and basal plates of the placenta, where $p < 0.001$.

To substantiate the obtained results, it is necessary to focus on the essence of histochemical method for free amino groups of proteins by the method of A. Yasuma and T. Ichikawa. The peculiarity of this technique is that it detects only free groups of proteins. Therefore, this technique shows the number of unhidden (free) amino groups of proteins rather than the total amount of protein. If there is a loss of proteins of a higher degree of structural organization (denaturation of proteins), the hidden amino groups of proteins become free - available for oxidation by ninhydrin [14]. Therefore, in order to be able to interpret the obtained data on limited proteolysis, there was conducted a quantitative determination of total

protein (using the method with bromophenol blue according to Bonheg) in fibrinoid of the chorionic and basal plate of the placenta in acute and chronic chorioamnionitis and basal deciduitis in case of IDA and without it. The results of the study are shown in table (Table III and Table IV).

When comparing these figures, it is obvious that in case of iron deficiency anemia of pregnant women without inflammation, as in all forms of inflammation of the manure, the results are on average statistically close to the placental physiological pregnancy. The same tendency is observed at a combination of an inflammation with IDA of pregnant women.

Therefore, if the total amount of protein does not change, which indicates the fact of sufficient regeneration of proteins in cells, and the color in the ninhydrin-Schiff reaction increases, it means that the protein loses its ability to perform its functions and denaturation of proteins appears. Thus, histochemical determination of limited proteolysis is one of the subtle methods of establishing one of the forms of alteration of structures.

In addition, the results obtained are important for a full understanding of free radical processes, which, according to various authors, are activated in both inflammation of the manure and iron deficiency anemia of pregnant women. Thus, the identified features of limited proteolysis complement the previously obtained and substantiated data on chemiluminescent study of nitroperoxides in placental structures [7] and processes of oxidative modification of proteins in the inflammatory focus [8] in combination with inflammation of manure and iron deficiency anemia of pregnant women, which reflects a logical connection between the processes: increase in nitroperoxides, followed by intensification of oxidative modification of protein activity, with increasing activity of limited proteolysis processes. This gives reason to believe that the key factor in the formation of morphological features of placental inflammation is the intensification of free radical processes, and iron deficiency additionally significantly modifies these processes, which in turn enhances proteolysis.

CONCLUSIONS

1. Iron deficiency anemia of pregnant women intensifies the processes of limited proteolysis according to the optical density of histochemical staining for free amino groups of proteins in the fibrinoid of the chorionic ($p < 0.001$) and basal plate ($p < 0.001$) of the placenta in comparison with physiological pregnancy.

2. In acute and chronic forms of chorioamnionitis ($p < 0.001$ / $p < 0.001$) and basal deciduitis ($p < 0.001$ / $p < 0.001$), the processes of limited proteolysis are intensified according to quantitative indicators of the optical density of histochemical staining in comparison with physiological pregnancy.
3. Comorbid iron deficiency anemia of pregnant women activates the processes of limited proteolysis only in the chronic form of chorioamnionitis ($p < 0.001$) and basal deciduitis ($p < 0.001$) in comparison with inflammation without anemia.

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ORCID and contributionship:

Vitalii V. Ilika: 0000-0002-3714-9883^{A-D}

Oleksandra V. Garvasiuk: 0000-0002-1936-2015^{B,C,E}

Oleksandra I. Dogolich: 0000-0002-5309-2602^E

Batih V. Iryna: 0000-0002-8498-921X^B

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The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Vitalii V. Ilika

Bukovinian State Medical University

2 Theatrical Sq., 58000 Chernivtsi, Ukraine

e-mail: vitaliy.ilika@bsmu.edu.ua

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