

HISTOCHEMICAL EVALUATION OF PROTEIN OXIDATIVE MODIFICATION IN FIBRINOID OF THE PLACENTA, DEPENDING ON CALCIUM DEPOSITS, COMBINED WITH IRON DEFICIENCY ANEMIA OF PREGNANCY

Andrii I. POPOVYCH^{1✉}, Igor S. DAVYDENKO¹, Oksana M. DAVYDENKO²

¹ Department of Pathological Anatomy, Bukovinian State Medical University (BSMU), Ukraine

² Department of infectious diseases and epidemiology, BSMU, Ukraine

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ABSTRACT

Introduction. Both calcium and iron significantly affect the level of free radical processes.

The aim of the study was to determine the levels of protein oxidative modification in fibrinoid of placenta of different localization areas, depending on the variants of calcium deposits, based on histochemical methods of bromophenol blue staining of proteins into „acidic“ and „basic“ by Mikel Calvo with quantitative assessment of results by means of computed microspectrophotometry.

Materials and methods. 164 cases of placental calcification, including 84 cases of iron deficiency anemia in pregnancy (IDAP) and 80 cases of pregnancy without anemia, were studied. Histochemical method of bromophenol blue into „acidic“ and „basic“ proteins by Mikel Calvo with the quantitative assessment of the staining results was performed by use of computed microspectrophotometry on the digital copy of the image with the fibrinoid areas in the computed program „ImageJ“.

Results. The differences between the average indicator R/B, which is the measure of protein oxidative

RÉSUMÉ

Evaluation histochimique des processus de modification de l'oxydation des protéines dans les fibroïdes du placenta dans sa calcification combinée avec l'anémie ferriprive des femmes enceintes

Introduction. Le calcium et le fer affectent significativement le niveau des processus des radicaux libres.

Le but de l'étude. Pour établir le niveau de modification oxydante des protéines dans le placenta fibroïde de localisation différente en fonction de la variante de dépôts de calcium sur la base de la technique histochimique avec du bleu de bromophénol aux protéines «aigres» et «basiques» par Mikel Calvo avec estimation quantitative des résultats de coloration par microspectrophotométrie.

Matériaux et méthodes. Ont été étudiés 164 cas de calcinose placentaire, y compris 84 cas d'anémie ferriprive et 80 cas de grossesse non anémiques. On a réalisé une technique histochimique avec du bleu de bromophénol pour les protéines «aigres» et «basiques» par Mikel Calvo avec une estimation quantitative des

✉ Corresponding author:

Andrii I. POPOVYCH

Department of Pathological Anatomy of the State Higher Educational Institution of Ukraine „Bukovinian State Medical University“, Ukraine

Teatralna Sq. 2, Chernivtsi, 58002, Ukraine

Phone +380506911693,

e-mail: andriy_popovych@ukr.net

modification, were discovered both in fibrinoid as a part of the chorionic tree and in the basal plate of the placenta in particular types of calcium deposits.

Conclusion. According to the histochemical study, pregnancies with iron deficiency anemia were characterized by fibrinoid with deposits of calcium type II and type IV (fine-granular deposits) both in chorionic tree and in the basal plate of the placenta, where the protein oxidative modification processes increase compared to those without anemia.

Keywords: calcium deposits, iron deficiency anemia in pregnancy, protein oxidative modification.

Abbreviations: IDAP – iron deficiency anemia in pregnancy, R/B – red/blue, Ob – objective, Oc – ocular.

INTRODUCTION

Calcification, liming or petrification (an increased level of insoluble calcium salts deposits) of the placenta is a fairly widespread phenomenon¹, and placental calcification can often be found in combination with anemia in pregnancy^{2,3}. Based on the previous studies^{4,5}, it was found that the frequency of distribution of the studied variants of calcium deposits is different in iron deficiency anemia in pregnancy (IDAP) compared with the placental calcification without anemia (that is, with normal blood parameters during pregnancy). Moreover, these differences pertain to fibrinoid of the placenta, both in the area of the chorionic tree as well as in the basal plate of the placenta. Since both calcium and iron significantly affect the level of free radical processes, the research was conducted to define the level of protein oxidative modification in fibrinoid of the placenta of different localization depending on the variant of calcium deposits based on the histochemical technique of bromophenol blue into „acidic“ and „basic“ proteins by Mikel Calvo with the quantitative evaluation of the staining results by means of computer micro spectrophotometry. The idea of the method is that bromophenol blue differentiates the „acidic“ and „basic“ proteins by means of certain staining techniques. The „basic“ proteins are dyed into blue, and the „acidic“ proteins are dyed into red, yellow and green colors. According to the original interpretation of Mikel Calvo (1957), „acidic“ proteins are the proteins characterized by the predominance of carboxyl groups over amino groups, and the „basic“ proteins are those in which amino groups predominate over

résultats de coloration par microspectrométrie informatique sur des copies numériques des sites fibrinoïdes dans le milieu du programme informatique Image].

Résultats de l'étude. Les différences dans les valeurs moyennes de l'indice R/B, qui est une mesure de la modification d'oxydation des protéines, sont trouvées dans les fibrinoïdes tant dans la zone de l'arbre chorionique que dans la plaque basale du placenta sur les différents types de dépôts de calcium.

Conclusion. Selon l'étude histochimique de l'anémie ferriprive chez les femmes enceintes présentant des dépôts fibrinoïdes de type II et IV (dépôts granulaires fins) dans la zone de l'arbre chorionique et dans la plaque basale du placenta, les processus de modification oxydante des protéines augmentent fortement par rapport aux observations sans anémie.

Mots-clés: dépôts de calcium, anémie ferriprive chez la femme enceinte, modification oxydante des protéines.

carboxylic groups. It should be mentioned that in biological tissues proteins are usually found as the constituent parts of „mixtures“. Therefore, when performing the Mikel Calvo staining technique, the coloration is predominantly characterized by smooth transitions both in terms of spectral characteristics and intensity. This is the reason for the application of quantitative research in our study.

The theoretical basis for the quantitative evaluation of the coloration by means of bromophenol blue is also that it dyes the proteins stoichiometrically, that is, the amount of dye corresponds to the amount of protein. Changes in the correlation between „acidic“ and „basic“ proteins should appear with the intensification of processes of protein oxidative modification. These processes are known to be characterized by oxidation of amino groups of amino acids of proteins, which leads to the violation of the correlation between functional groups of proteins in favor of carboxyl groups. The application of the Mikel Calvo technique, therefore, will be marked by the predominance of the red component of the spectrum.

Fibrinoid of the placenta is a convenient research object for proteins, because it contains a large number of them. At the same time, fibrinoid is a sufficiently stable object, which allows it to make extrapolations in a process such as placental calcification, in particular.

THE AIM OF THE STUDY was to define the levels of protein oxidative modification in fibrinoid of the placenta of different localization areas, depending on the version of calcium deposits, based on the histochemical technique of bromophenol blue into

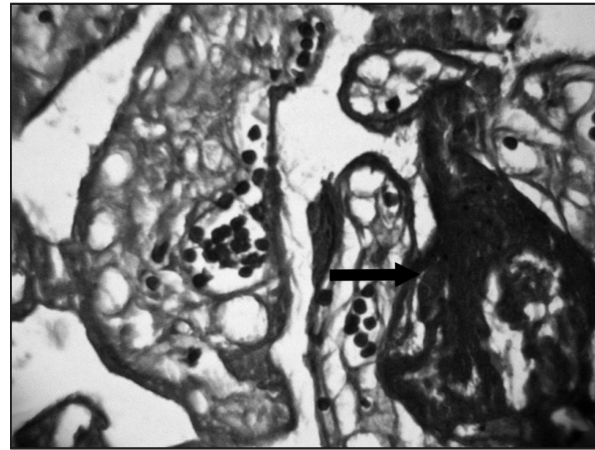
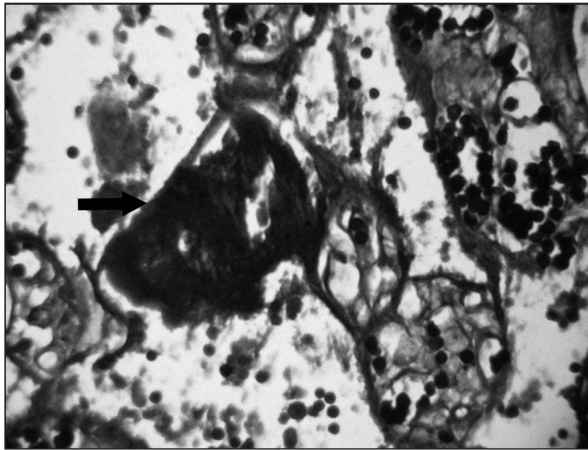


Figure 1. Intervillous fibrinoid in placental calcification. Fibrinoid cells are indicated by arrows. Bromophenol blue staining by Mikel Calvo. Ob 10^x. Oc 10^x

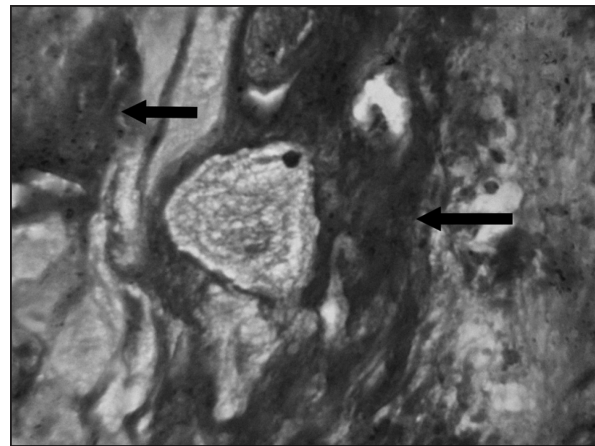
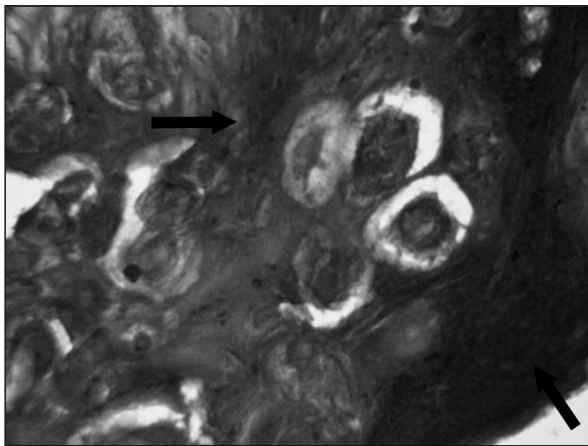


Figure 2. Fibrinoid of the basal plate of placenta in its calcification. Fibrinoid cells are indicated by arrows. Bromophenol blue staining by Mikel Calvo. Ob 40^x. Oc 10^x

„acidic“ and „basic“ proteins by Mikel Calvo, with the quantitative evaluation of these results by means of computer microspectrophotometry.

MATERIALS AND METHODS

164 placentae with calcification were studied, the duration of pregnancy was 29-40 weeks. 84 pregnant women were diagnosed with IDAP (I-II severity scale), including 40 pregnant with preterm labour and 44 pregnant with urgent labour. The rest of the pregnancy observations (80) had no signs of anemia and included 38 pregnant with preterm labour and 42 pregnant with urgent labour. The calcification of the placenta was diagnosed only in those cases when in material sampled from different cotyledons the deposits of calcium were found in at least four cotyledons simultaneously. The material was placed in neutral buffered formalin for 24 hours. After being dehydrated in a battery of ethanol, the

slices of the placenta were embedded with paraffin. The sections of 5 microns thick were made on the sliding microtome. The histochemical technique of bromophenol blue into „acidic“ and „basic“ proteins by Mikel Calvo in its own modification⁶ was performed on deparaffinized histological sections with the quantitative evaluation of the staining results by computer micro spectrophotometry on digital copies of fibrinoid areas by means of the computer program ImageJ (1.48v, free license, W. Rasband, National Institute of Health, USA, 2015⁷). The final result of the computer micro spectrophotometry was R/B index, which served as a measure of protein oxidative modification. The arithmetic mean and its error were calculated for each study group. The Shapiro-Wilk criterion was used to verify normality in the statistical samples. The differences in the average tendencies were estimated using the unpaired two-sample Student's test. All statistical calculations were performed in the computer program PAST v3.14, a free license, O. Hammer, 2016⁸.

RESULTS, DISCUSSION

In general, seven morphological variants of calcium deposits were studied in two localization areas⁴. The differences were observed in the chorial placental tree in the distribution frequency depending on the presence of IDAP for types II, III, IV of calcium deposits. In the basal plate there were found only for types II and IV. Type II is a combination of multiple fine-grained dust-like groups of calcium deposits. They can be found in different parts of the fibrinoid bodies and evenly „mixed“ with fibrinoid throughout its volume. Individual fine-grained dust-like calcium deposits that do not form a group are considered to be a subspecies of this kind. These deposits are relatively badly stained. Type III belongs to a large plate-like group of deposits, which is usually not stained very intensively, although there are some exceptions to this rule. These deposits can be localized in any part of the fibrinoid body, often occupying its main part. Type IV is a peculiar combination of the plate-like and fine-grained deposits described above. The peculiarity consists in the patterns of their mutual arrangement i.e. plate-like structures are always located in the center, while fine-grained structures are located along their periphery. These deposits are always large in size and they occupy a significant part of the fibrinoid body. It is the fibrinoid with the abovementioned types of calcium deposits that has been analyzed in detail on the subject of protein oxidative modification.

The arithmetical mean of the R/B index in fibrinoid in the area of the chorial placental tree,

depending on the morphological variants of calcium deposits, are given in Table 1. According to the data given, in IDAP in the intervillous fibrinoid with calcium deposits of type II, the processes of protein oxidative modification are increasing in comparison with those without anemia. The same pattern has been observed for the intervillous fibrinoid with calcium deposits – Type IV. At the same time, there was no statistical discrepancy in the average tendencies for the R/B index in the intervillous fibrinoids with calcium deposits – Type III.

The arithmetical means of the R/B index in the area of the placental basal plate are given in Table 2. It can be inferred from these data that in IDAP in the basal plate of fibrinoid, as well as in the area of the chorial tree in fibrinoid with calcium deposits – type II and type IV, the processes of protein oxidative modification sharply increase compared to the observations without anemia.

As calcium deposits of type II and type IV are characterized by small granularity, and those of type III are characterized by plate-like nature of calcium salts deposition, it can be assumed that the granular nature of calcium deposits reflects the period of their formation, it indicates that these deposits are relatively „fresh“, while plate-like deposits, on the contrary, were formed earlier. To define the specific time intervals an individual research will be required.

The obtained results allow us to assume the following working hypothesis regarding the development mechanism of the revealed phenomena. Iron deficiency anemia in pregnancy leads to the increased

Table 1. R/B index in fibrinoid in the area of the chorial placental tree depending on the morphological variants of calcium deposits.

<i>Morphological variant of calcium deposits</i>	<i>Observation of iron deficiency anemia in pregnancy (I-II severity scale)</i>	<i>Observation of women with normal blood tests during pregnancy</i>
Deposits in the intervillous fibrinoid – type II	2.24±0.108 n=81	1.34±0.112 n=22 p<0,001
Deposits in the intervillous fibrinoid – type III	1.28±0.145 n=38	1.22±0.121 n=64
Deposits in the intervillous fibrinoid – type IV	2.22±0.114 n=70	1.26±0.128 n=54 p<0,001

Table 2. R/B index in fibrinoid of the basal plate of the placenta depending on the morphological variants of calcium deposits.

<i>Morphological variant of calcium deposits</i>	<i>Observation of iron deficiency anemia in pregnancy (I-II severity scale)</i>	<i>Observation of women with normal blood tests during pregnancy</i>
Deposits in fibrinoid of the basal plate – type II	2.21±0.104 n=78	1.28±0.145 n=24 p<0,001
Deposits in fibrinoid of the basal plate – type IV	2.16±0.119 n=49	1.20±0.138 n=33 p<0,001

damage of maternal erythrocytes in the interstitial spaces of the placenta and hypoxic alteration of the trophoblast, which enhances the processes of formation of intervillous fibrinoid and fibrinoid of the basal plate.

The processes of placental calcification, which are most likely caused by cell death, are increasing simultaneously. The growth of the intensity of free radical processes in maternal blood, which usually accompanies anemic conditions and iron deficiency, causes the intensification of the protein oxidative modification. This is most pronounced in fibrinoid of the placenta.

CONCLUSION

According to the histochemical study, pregnancies with iron deficiency anemia are characterized by fibrinoid with calcium deposits type II and type IV (fine-granular deposits), both in the chorial tree and in the basal plate of the placenta, where the protein oxidative modification processes increase compared with the observations without anemia.

Prospect of further research. The area of our further research is the histochemical evaluation of the processes of limited proteolysis in the localizations under study.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in 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“

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