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## DISTRIBUTION OF MORPHOLOGICAL VARIANTS OF CALCIUM DEPOSITS IN PLACENTA OF GRAVIDAS WITH IRON-DEFICIENCY ANEMIA

**Keywords:** *calcium deposits, iron-deficiency anemia.*

**Abstract.** *Our research conducted an investigation of 164 observations of placental calcinosis, including 84 observations of gravidas with iron-deficiency anemia and 80 observational gravidities without anemia. Frequency analysis of different variants of calcium deposits in chorial placental tree showed that type II and type IV perivillous fibrin deposits strongly dominated in gravidas with iron-deficiency anemia (over 90% and 80% placentas respectively). There were also deposits found in type III perivillous fibrin (about 45% of cases). These indices significantly exceeded the values of the frequency of women whose anemia had not been recorded during gravidity. Other variants of calcium deposits in iron-deficiency anemia occurred much more rarely. It should be mentioned, though, that in cases accompanied by gravidas with iron-deficiency anemia in comparison with observations of gravidas without anemia small granular deposits were more commonly found in the placental plasmodium of the chorial villi. Frequency analysis of different options of calcium deposits in the basal plate of the placenta showed that the observations of gravidas with iron-deficiency anemia as well as the observations of those without anemia dominated by calcium deposits in fibrin of all four types (type IV slightly smaller compared to other types). However, it should be pointed out that type II and type IV fibrin deposits were more frequently found in gravidas with iron-deficiency anemia compared with the observations without anemia ( $P < 0,05$ ).*

### Introduction

The study deals with elucidation of new data concerning a quantitative distribution of morphological variants of calcium deposits in placentas with calcinosis in case of gravidas with iron-deficiency anemia as compared to the placentas with calcinosis without anemia (that is, with normal hematological signs during pregnancy).

The study is initiated by the findings concerning increased occurrence of calcinosis among pregnant women with severe forms of gravidas with iron-deficiency anemia [1] and is based on a working hypothesis that metabolic changes occurring due to iron deficiency and the whole complex of changes in the blood of gravidas with iron-deficiency anemia might affect the morphology of calcium deposits which can be further used for diagnostic purpose.

Only those observations were thought to be afflicted with placental calcinosis when during material sampling from different cotyledons calcium deposits were regularly found no less than in four cotyledons [2].

**Objective of the study:** to detect a quantitative

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distribution of morphological variants of calcium deposits in placenta in case of gravidas with iron-deficiency anemia as compared to the observation of pregnancy without anemia.

### Material and methods

Morphological peculiarities of calcium deposits were described on the basis of staining techniques of histological sections with hematoxylin with additional weak supplemental staining with eosin (for better contrast of deposits). Calcium nature of the deposits was proved by serial sections on the basis of histochemical methods with alizarin red.

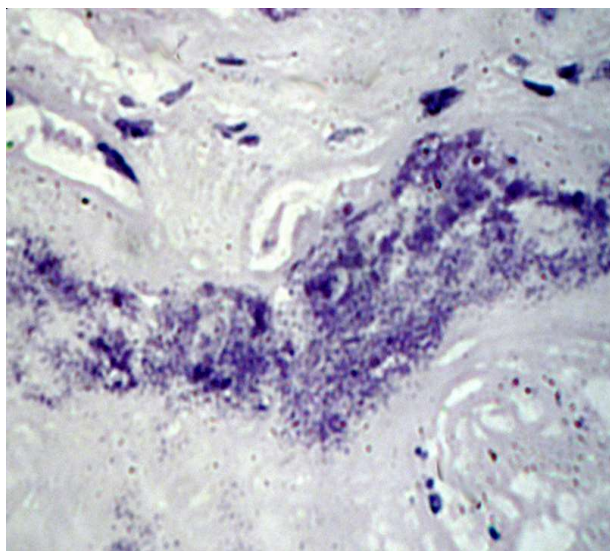
164 placentas with the terms of gestation 29-40 weeks were examined, at the same time the diagnosis of iron-deficiency anemia (I-II degree of severity) was made in 84 pregnant women (including 40 of those with preterm labour, 44 - term labour), the rest of observations of pregnancy was without anemia - 80 altogether (38 - preterm labour, 42 - term labour).

Absolute values of occurrence and percentage of different variants of calcium deposits were

calculated. Difference probability between the percentage of the examined groups was checked by means of binomial method as the most accurate and adequate one for the purposes of paired comparison of frequencies. The calculations according to binomial method were performed by means of the computer program PAST: Paleontological Statistics, Version 3.14. (? Hammer, 2016) [3]. In order to present the information completely the percentage was given together with metering error which is an estimating value and depends on both the percentage itself and general number of observations.

### Results and discussion

Histological study of the placentas examined demonstrated that in case of term labour there was no hypermaturity found [2], in case of preterm labour there was no preterm maturation of chorionic tree [4] or uterine-placental area found [5], that could be causes of placental calcinosis. Therefore, etiology of calcinosis studied did not relate to preterm maturation



**Fig.1.** The part of placental fibrinoid with calcium deposits. Calcium deposits are of a granular structure (indicated with arrows). Hematoxylin and eosin staining. Ob.40<sup>x</sup>. Oc.10<sup>x</sup>

themselves and the whole deposit. Such deposits are always located deep in fibrinoid bodies.

Type II - multiple fine-grained dust-like groups of calcium deposits. They can be in different parts of fibrinoid bodies as well as evenly "mixed" with fibrinoid along its whole volume. Separate fine-grained dust-like calcium deposits which do not form groups are considered to be a subtype of this type. Such kinds of deposits are stained comparatively weakly.

Type III - large lamellar deposits as a rule stained not very intensively, although there are some exceptions from the rule. These deposits can be localized in any part of the fibrinoid body often

of the placenta.

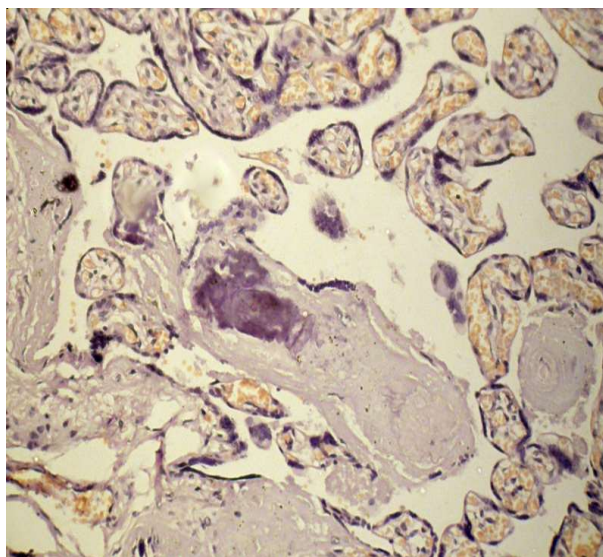
In histological sections of the placenta calcium deposits were found both in the area of the chorionic tree and basal lamina.

A part of deposits was not associated with placental fibrinoid, while another part was in different ways involved into placental fibrinoid. Calcium deposits were evaluated according to the principles elaborated before [6].

Those deposits not involved into fibrinoid were either of fine granular (Fig.1) or lamellar character (Fig.2), and sometimes they were of a combined character and differed by their localization.

Preliminary conducted studies of calcium deposits involved into placental fibrinoid enabled to differentiate the following four variants (types) of deposits [7], described in this article with certain new details.

Type I - comparatively fine but at the same time gross intensively stained calcium deposits in the form of large heterogeneous structures clearly outlined



**Fig.2.** The part of placental fibrinoid with calcium deposit. Calcium deposit of a lamellar structure (indicated with an arrow). Hematoxylin and eosin staining. Ob.20<sup>x</sup>. Oc.10<sup>x</sup>

occupying its whole volume.

Type IV - a distinctive combination of lamellar and fine-grained deposits described above. Their singularity is contained in the regularity of their mutual location - lamellar structures are always located in the center, fine-grained ones - in their periphery. These deposits are always of large sizes, they occupy a considerable volume of a fibrinoid body.

It should be noted that in every separate placenta certain deposits are always found referring to different variants, therefore the data presented in Table 1 and 2 should be considered as combinatorial ones but not alternative. At the same time it should

Table 1

**Absolute frequency and percentage of morphological variants of calcium deposits in chorionic tree of the placenta**

<b>Morphological type of calcium deposits</b>	<b>Observation of iron-deficiency anemia of pregnancy (I-II degree of severity) n=84</b>	<b>Observation of women with normal blood analysis during pregnancy n=80</b>
Fine granular deposits in syncytiotrophoblast	8 (9,5±3,20%)	1 (1,2±1,21%) P=0,034
Fine granular deposits in the stroma of chorionic villi	6 (7,1±2,81%)	5 (6,2±2,70%)
Large lamellar deposits in the stroma of chorionic villi	1 (1,2±1,19%)	2 (2,5±1,81%)
Deposits in inter-villous fibrinoid – type I	14 (16,7±4,10%)	15 (18,8±4,41%)
Deposits in inter-villous fibrinoid – type II	81 (96,4±2,00%)	22 (27,5±5,01%) P<0,001
Deposits in inter-villous fibrinoid – type III	38 (45,2±5,50%)	64 (80,0±4,52%) P=0,003
Deposits in inter-villous fibrinoid – type IV	70 (83,3±4,10%)	54 (67,5±5,31%) P=0,031

Note. The percentage between the groups of observation was compared by means of binomial method. In case of probable difference on the level (P<0,05) its specific value is presented in the Table, in case this level of probability is not reached the value P is not presented.

Table 2

**Absolute frequency and percentage of morphological variants of calcium deposits in basal lamina**

<b>Morphological type of calcium deposits</b>	<b>Observation of iron-deficiency anemia of pregnancy (I-II degree of severity) n=84</b>	<b>Observation of women with normal blood analysis during pregnancy n=80</b>
Fine granular deposits in cytotrophoblast	2 (2,4±1,71%)	2 (2,5±1,81%)
Fine granular deposits in the stroma outside fibrinoid	5 (5,9±2,49%)	4 (5,0±2,50%)
Large lamellar deposits in the stroma outside fibrinoid	2 (2,4±1,71%)	2 (2,5±1,81%)
Deposits in basal lamina fibrinoid – type I	74 (88,1±3,59%)	70 (87,5±3,70%)
Deposits in basal lamina fibrinoid – type II	78 (92,9±2,80%)	24 (30,0±5,19%) P<0,001
Deposits in basal lamina fibrinoid – type III	68 (80,9±4,32%)	64 (80,0±4,52%)
Deposits in basal lamina fibrinoid – type IV	49 (58,3±5,40%)	33 (41,2±5,50%) P=0,043

Note. The percentage between the groups of observation was compared by means of binomial method. In case of probable difference on the level (P<0,05) its specific value is presented in the Table, in case this level of probability is not reached the value P is not presented.

be noted that variability of deposit variants in every separate placenta as a rule did not involve more than two-three variants.

Absolute frequency and percentage of morphological variants of calcium deposits in chorionic tree are presented in Table 1.

The data given in the Table 1 are indicative of the

fact that in case of gravidas with iron-deficiency anemia deposits of type II and type IV prevail in the inter-villous fibrinoid in chorionic tree (over 90% and 80% respectively). In addition, deposits of type III are often found in the inter-villous fibrinoid (about 45% of observations). The indices indicated increased considerably (P<0,05) the values of frequencies in the

group of women without anemia during pregnancy. Other variants of calcium deposits in case of gravidas with iron-deficiency anemia are found less frequently, although it should be noted that in case of gravidas with iron-deficiency anemia as compared to the pregnancies without anemia fine granular deposits are found more frequently in the syncytiotrophoblasts of the chorionic villi of the placenta ( $P < 0,05$ ). Those villi were of different types, although this phenomenon was more often found in mature variants of the villi - intermediate mature, terminal, terminal "specialized", and stem "late" villi [4].

Absolute frequency and percentage of morphological variants of calcium deposits in basal lamina are presented in Table 2.

The data presented are indicative of the fact that calcium deposits of all four types prevail in fibrinoid in the basal lamina both in case of gravidas with iron-deficiency anemia and without anemia (type IV is found a little less as compared to the other types). At the same time, it should be noted that in case of gravidas with iron-deficiency anemia deposits of type II and type IV are found more often than those in comparison with cases without anemia ( $P < 0,05$ ).

### Conclusions

1. Analysis of frequency of different variants of calcium deposits in the chorionic tree of the placenta are indicative of the fact that in case of gravidas with iron-deficiency anemia deposits of type II and type IV prevail in the inter-villous fibrinoid in chorionic tree (over 90% and 80% respectively). In addition deposits of type III are often found in the inter-villous fibrinoid (about 45% of observations). The indices indicated increased considerably ( $P < 0,05$ ) the values of frequencies in the group of women without anemia during pregnancy. Other variants of calcium deposits in case of gravidas with iron-deficiency anemia are found less frequently, although it should be noted that in case of gravidas with iron-deficiency anemia as compared to the pregnancies without anemia fine granular deposits are found more frequently in the syncytiotrophoblasts of the chorionic villi of the placenta ( $P < 0,05$ ).

2. Analysis of frequency of different variants of calcium deposits are indicative of the fact that calcium deposits of all the four types prevail in fibrinoid in the basal lamina both in case of gravidas with iron-deficiency anemia and without anemia (type IV is found a little less as compared to the other types). At the same time, it should be noted that in case of gravidas with iron-deficiency anemia deposits of type II and type IV are found more often than those in comparison with cases without anemia ( $P < 0,05$ ).

### Perspectives of further studies

Further perspective directions of the study are those concerning detection of the mechanisms of the development of different variants of calcium deposits in case of iron-deficiency anemia of pregnancy.

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### РАСПРЕДЕЛЕНИЕ МОРФОЛОГИЧЕСКИХ ВАРИАНТОВ ОТЛОЖЕНИЙ КАЛЬЦИЯ В ПЛАЦЕНТЕ ПРИ ЖЕЛЕЗОДЕФИЦИТНОЙ АНЕМИИ БЕРЕМЕННЫХ

*А.И. Попович, И.С. Давыденко, А.В. Гарвасюк*

**Резюме.** Изучено 164 случая кальциноза плаценты, в т.ч. 84 случая железодефицитной анемии (ЖДАБ) и 80 случаев беременности без анемии. Анализ частоты различных вариантов отложений кальция в хориальном дереве плаценты показал, что при железодефицитной анемии беременные резко преобладают отложения в межворсинчатом фибриноиде типа II и типа IV (более у 90% и 80% плацент соответственно). Также при ЖДАБ часто обнаруживают отложения в межворсинчатом фибриноиде типа III (около 45% случаев). Указанные показатели существенно превышают величины частот группы женщин, у которых не отмечалось анемии во время беременности. Другие варианты отложений кальция при ЖДАБ встречаются значительно реже, хотя следует отметить, что при ЖДАБ по сравнению с наблюдениями беременности без анемии, чаще встречались мелкие гранулярные отложения в синцитиотрофобласте хориальных ворсинок плаценты. Анализ частоты различных вариантов отложений кальция в базальной пластинке плаценты показал, что, как в наблюдениях ЖДАБ, так и в наблюдениях без анемии преобладают отложения кальция в фибриноиде всех четырех типов (чуть меньше типа IV по сравнению с другими типами). Вместе с тем, следует указать на то, что при ЖДАБ по сравнению с наблюдениями беременности без анемии все же чаще ( $P < 0,05$ ) обнаруживают отложения фибриноида типа II и типа IV.

**Ключевые слова:** отложение кальция, плацента, железодефицитная анемия беременных.

### РОЗПОДІЛ МОРФОЛОГІЧНИХ ВАРІАНТІВ ВІДКЛАДАНЬ КАЛЬЦІУ У ПЛАЦЕНТІ ПРИ ЗАЛІЗОДЕФІЦИТНІЙ АНЕМІЇ ВАГІТНИХ

*А.І. Попович, І.С. Давиденко, О.В. Гарвасюк*

**Резюме.** Вивчено 164 спостереження кальцинозу плаценти, у т.ч. 84 спостереження залізодефіцитної анемії (ЗДАВ) та 80 спостережень вагітності без анемії. Аналіз частоти різних варіантів відкладань кальцію в хоріальному дереві плаценти показав, що при ЗДАВ різко переважають відкладання в міжворсинчастому фібриноїді типу II та типу IV (понад у 90% та 80% плацент відповідно). Також при ЗДАВ часто виявляють відкладання в міжворсинчастому фібриноїді типу III (біля 45% спостережень). Вказані показники суттєво перевищують величини частот групи жінок, у яких не відмічалось анемії під час вагітності. Інші варіанти відкладань кальцію при ЗДАВ зустрічаються значно рідше, хоча слід відмітити, що при ЗДАВ, у порівнянні зі спостереженнями вагітності без анемії, частіше зустрічалися дрібні гранулярні відкладання в синцитіотрофобласті хоріальних ворсинок плаценти. Аналіз частоти різних варіантів відкладань кальцію в базальній

пластинці плаценти показав, що, як у спостереженнях залізодефіцитної анемії вагітних так і в спостереженнях без анемії переважають відкладання кальцію в фібриноїді всіх чотирьох типів (трохи менше типу IV в порівнянні з іншими типами). Разом з тим, слід вказати на те, що при залізодефіцитній анемії вагітних у порівнянні зі спостереженнями вагітності без анемії все ж частіше ( $P < 0,05$ ) виявляють відкладання фібриноїду типу II та типу IV.

**Ключові слова:** відкладання кальцію, плацента, залізодефіцитна анемія вагітних.

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