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The morphological analysis of the haematopoietic microenvironment of the bone marrow at foetuses from preterm pregnancies

During the perinatal period the hemodynamic processes (foetal circulation changes into the circulation of the adult type) are accompanied by alterations in organs participating in haematopoietic functions. It is when both the mother and the foetus show sporadic disorders of the coagulation system. In extreme cases dissaminate intravascular coagulation syndrome is observed, which may constitute a direct threat to life in both the foetus and the mother. Bone marrow is the main haematopoietic organ in adult mammals (3, 8, 10).

During foetal period and infancy the haematopoietic functions occur also in liver, spleen, sporadically in other inner organs and in placenta. Foetuses show morphological or functional underdevelopment of various organs and systems with respect to the duration of the gestation (5, 6, 7).

The aim of the study was morphological analysis of the haematopoietic microenvironment of the bone marrow at foetuses from preterm pregnancies.

OBJECTIVE AND METHODS

The study was carried out on 21 foetuses selected at the Department of Clinical Pathomorphology of the Medical University of Białystok. The foetal age ranged from 24th to 34th week of gestation. For the morphological assessment the bone marrow was taken from sternum at intercostals space II and was fixed with the so-called "Oxford" fixing solution for 24 hours. After the fixation, fragments of the bone marrow of 1x 0.5x 0.2 cm size were embedded in paraffin, from which the microscopic sections 5 mm thick were cut with using the sledge microtome. Immunohistochemical methods were used to identify young cellular lines of megakaryocytary line. Detailed evaluation of bone marrow stroma was performed on the basis of configuration of the vascular sinuses, megakaryocytes and their participation in creating the bone marrow – blood barrier, system of osteal trabeculae amount of argentophilic fibers and distribution of $\alpha 2\ \beta 1$ integrin. Demonstration and assessment of argentophilic fibres amount was carried out using the impregnation with silver according to Gomori. The configuration of the vascular sinuses was defined with morphometric evaluation of vascular space with MicroImage-Olympus Kit. Identification of the 'young vascular forms' and cellular forms was performed by immunohistochemical methods with the use of endothelial cell antibodies and factor VIII. Identification of the 'young vascular forms' was performed with immunohistochemical methods with the use of the endothelial cell antibodies CD 31 (clone JC/70A) and factor VIII (clone F8/86), antibodies CD 41 (clone 5B12) defining platelal glicoprotein IIb as well as antibodies CD 61 (clone Y2/51) defining platelal glycoprotein IIIa. Anti-integrin antibodies (clone P1E6) were used to identify integrin. As detection kits LSAB+system HRP kit was used and DAB as chromogene (DAKO).

RESULTS

Integrin was found in individual megakaryocytes, endothelial cells of sinusoidal vessels and the cells forming the stroma. Integrin presence in these cells may indicate their immaturity. With respect to foetal age variable frequency and intensification of integrin reaction in the examined elements of bone marrow were observed. The younger the foetus, the weaker intensification of the reaction in stroma cells. The reaction was stronger in individual megakaryocytes and endothelial cells. The configuration of blood vessels vascular sinuses was assessed as well as their mutual proportions. In preparations of younger foetuses fine blood vessels were more frequently observed than vascular sinuses, while in older foetuses the relation was reversed. The more advanced development of the foetus the larger configuration of vascular sinuses stroma in bone marrow (Table 1). Another observed phenomenon was more frequent occurrence of megakaryocytes in direct vicinity of sinusoidal vessels in younger foetuses. These megakaryocytes usually develop a weak reaction with integrin. However, no differences were found in the quantity of argentophilic fibres.

Table 1. Vessels surface

Examined group	N	X	SD	min-max	Relation of the sinus vessels surface to bone marrow surface (%)
I (24–26 gestation week)	5	532.27	243.34	141.21-1098.11	8.8
II (27–28 gestation week)	5	806.98	595.65	280.68-2138.4	6.6
III (29–31 gestation week)	5	552.24	356.68	277.52-2143.08	10.3
IV (32–34 gestation week)	6	566.24	592.62	110.94-2416.03	12.3

DISCUSSION

The study revealed that in ontogenetic development of human haematopoietic tissue there are not only changes connected with the process of cellular maturation and differentiation, but also topographic alterations and mutual relation of individual cells (4, 7, 9). The mutual influence of microenvironment and cells has not been completely explored yet. Studies revealed that myeloma cells contain different receptors for individual proteins of extracellular space, which is probably connected with more intensive proliferation of these cells (1, 2). The role of the phenomenon observed in bone marrow of the examined foetuses is quite obscure. Further research should reveal the mechanism of interactions between the cells and extracellular matrix.

CONCLUSIONS

1. The more advanced development of the foetus the lesser that of integrin.

2. The more advanced development of the foetus the larger configuration of vascular sinuses stroma in bone marrow.

3. The ontogenetic development of haematopoietic tissue not only consists in alterations connected with the maturing process and cellular differentiation, but in topographic changes as well.

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SUMMARY

The aim of the study was morphological assessment of the haematopoietic microenvironment of the bone marrow in foetuses from preterm pregnancies. For the morphological assessment the bone marrow was taken from sternum during an autopsy examination. After standard preparation, cellular elements of individual developmental lines were identified with the application of immunohistochemical methods. Evaluation of the bone marrow stroma elements was performed on the basis of configuration of the vascular sinuses, connective tissue elements, mutual topographic relations and integrin occurrence. The assessment of the argentophilic fibres was carried out with impregnation with silver according to the Gomori method. The configuration of the vascular sinuses was defined with morphometric evaluation with MicroImage-Olympus Kit. Identification of the 'young vascular forms' was performed with immunohistochemical methods with the use of endothelial cell antibodies and factor VIII. The presence of integrin was discovered in individual megakaryocytes, endothelial cells of sinus vessels and singular

cells forming a stroma. However, no differences were found in the quantity of argentophilic fibres. The study revealed that ontogenetic development of haematopoietic tissue not only consists in alterations connected with the maturing process and cellular differentiation, but in topographic changes as well.

Analiza morfologiczna mikrośrodowiska hematopoetycznego płodów z ciąż niedonoszonych

Celem pracy była ocena morfologiczna mikrośrodowiska hematopoetycznego szpiku u płodów z ciąż niedonoszonych. Szpik kostny do oceny morfologicznej pobrano z mostka w czasie badania autopsyjnego. Po typowym przygotowaniu identyfikowano elementy komórkowe poszczególnych linii rozwojowych, z wykorzystaniem metod immunohistochemicznych. Ocenę elementów podścieliska szpiku przeprowadzono na podstawie układu naczyń, elementów tkanki łącznej, wzajemnych stosunków topograficznych oraz występowania integryny. Do oceny ilości włókien srebrochłonnych w szpiku kostnym wykonano srebrzenie według Gomoriego. Układ zatok naczyniowych określono poprzez ocenę morfometryczną z wykorzystaniem zestawu MicroImage-Olympus. Identyfikację „młodych form naczyniowych" przeprowadzono metodami immunohistochemicznymi z wykorzystaniem przeciwciał endothelial cell i czynnika VIII. Stwierdzono obecność integryny w pojedynczych megakariocytach, komórkach endotelialnych naczyń zatokowych i pojedynczych komórkach tworzących zrąb. Nie stwierdzono natomiast różnic w ilości włókien srebrochłonnych. Z przeprowadzonych badań wynika, że w rozwoju ontogenetycznym tkanki krwiotwórczej dochodzi nie tylko do zmian związanych z procesem dojrzewania i różnicowania komórkowego, ale też do zmian topograficznych.