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Some Features of Structural Change of Cerebral Microvessels in Diabetes

ABSTRACT

To identify specificity of microvascular damage of the brain and a degree of structural abnormalities of vessel walls in diabetes (D) types 1 and 2 the morphological, morphometric and immunohistochemical research of autopsy cerebellum of patients with diabetes mellitus has been conducted. The structural change of brain microvessels in diabetes is noted to be a long-term process and characterized by low levels of expression of proliferation markers and apoptosis in fibrosis and hyalinosis according to the immunohistochemical study. The staging of microvascular damage of the brain in DM is defined, 3 morphological stages are allocated.

Keywords*:* diabetes mellitus, microangiopathy, brain, pathologic changes.

INTRODUCTION

The most characteristic feature of diabetes mellitus (DM) is the development of microangiopathy, frequency of which is 62-89% according to different authors [4]. Trigger mechanisms of its development are the genetic defects of the vascular wall, disorders of hormonal regulation, breach of the rheological properties of blood, tissue hypoxia, overproduction of hormones of insulin antagonists. The nature and the severity diabetic microangiopathy is determined by a combination of all these factors with specific metabolic disorders [1]. Microcirculatory disturbances have a leading role in the development of late complications of diabetes.

Chronic hyperglycemia ha s great impact on on the development of diabetic neuropathy [3]. The significance of hyperglycemia is determined by identical frequency of neuropathy in patients with diabetes type 1 and 2, although the pathogenesis of these forms of diabetes is different [1]. Symptomatic neuropathy is more common in people with poorly controlled diabetes [1,2].

Dysfunction of the nervous system occurs in parallel duration of diabetes and severity of metabolic disturbance. Long-term compensation for diabetic neuropathy improves and helps to reduce the frequency of this complication. This is evidenced by the results of a multicenter study «The Diabetes Control and complications Trial» (DCCT) [1, 2].

The life expectancy of patients with diabetes is increasing due to the optimization of monitoring and correction of blood glucose that promotes to an increase in its late complications. Nervous system takes a leading place among them. To date, diabetic encephalopathy is the least explored area of neurodiabetology.

MATERIALS AND METHODS

Comparative characteristic of morphological changes of brain microvessels was performed on autopsy material of patients with diabetes type 1 - 15 observations and diabetes type 2 – 30 observations. The comparison group included 10 patients who died with hyperglycemia on the background of intoxication of different genesis (pneumonia, peritonitis). The average age of patients with diabetes type 1 was 34 years, the average duration of the clinical manifestations of the disease was 12 years. The average age of patients with diabetes type 2 was 56 years, the average duration of the clinical manifestations of the disease was 16 years. The average age of patients with hyperglycemia due to severe intoxication was 34 years.

Methods. 1. Morphological methods: The study of the cerebellum sectional preparations stained with hematoxylin and eosin, Masson tri-color method, PAS-reaction.

2. Immunohistochemical study: Indirect immuno-peroxidase method with visualization system DAKO EnVision (Denmark) proliferation markers (PCNA, Ki67) and activation of apoptosis marker p53.

3. Morphometric method: a) measurement of the diameter of microvessels DCIRCLE (2√~AREAF / π); b) form factor (degree of deformation of blood vessels) FCIRCLE (4πAREAF / PERIMCROFT2); c) the thickness of the vascular wall (middle index of 10 measuring of wall thickness), was performed using a microscope with a video camera Axioplan 2 DXC-151A (Sony, Japan) by computer analysis of a digital image using a KS 200 software package (Kontron Electronic, Germany).

4. The statistical method of assessing the results of research using the applications of statistics.

RESULTS AND DISCUSSION

We observed a significant thickening of the walls of the arterioles and the bundle with irregular accumulation of PAS-positive substances in the basement membrane of microvessels with the deformation of the lumen by microscopic examination. There is a local increase in the number of pericytes in myo-adventitial layer, the accumulation in the thickened vascular wall

collagen fibers, which is a sign of a perivascular fibrosis, impregnation of wall plasma proteins leads to hyalinosis microvessels at the end. We noted the formation of single microaneurysms different shapes. Perivascular edema varying degrees of severity and spongiosis of tissue of the brain was determined around the modified microvessels, which is a manifestation of stagnation. We found that the morphological changes of microvessels in diabetes mellitus type 1 and type 2 have similar manifestations, but their degree of varies.

Diabetic microangiopathy can be divided into 3 stages according to light microscopy:

1) reversible stage of suffusion with proteins and lipids of blood plasma (a microscopic phenomenon bundles and thickening of the walls of blood vessels);

2) stage of local increase in the number of pericytes and fibrosis of thickened vessel wall;

3) irreversible stage of lipohyalinosis of vascular wall.

Glucose in high concentrations has direct toxic effects on cellular structures of vessels, which affects the morphology features of proliferation and activation of these structures in patients with CD type 1 and type 2. Proliferative activity of cellular structures of microvessels in DM was determined by the level of expression of PCNA and Ki67. Proliferating cell nuclear antigen (PCNA) is a subunit of DNA polymerase. The maximum level of PCNA observed during the S phase of the cell cycle when it forms a complex with an inhibitor of p21 [5]. The expression level of PCNA in diabetes type 1 was assessed as very low and determined in nuclei isolated pericytes of cerebellar arteriolar with fibrosis (Figure 1).

Ki67 is a nucleoprotein, which is expressed in all cell cycle phases except G0. Expression starts in late G1, peaking in the mitotic phase of the cycle [5]. The expression of the proliferation marker Ki67 was defined as negative in microvessel cells of the cerebellum in diabetes type 1.

The damage level of vascular endothelium was evaluated by detecting a marker of apoptosis p53. P53 protein is a major determinant of the cellular mechanism that leads to programmed cell death [5]. P53 expression was assessed as very low in the cells of the cerebellum microvessels in diabetes type 1 and was determined in individual endothelial cells (Figure 2).

The low level of expression of the proliferation marker PCNA in cells of hyalinized microvessel of the cerebellum was noted, in the presence of its expression in perivascular microglia and oligodendroglial nuclei in DM type 2. Often, the expression of PCNA in cells hyalinized precapillaries was absent (Fig 3, 4).

The expression of the proliferation marker Ki67 in microvascular endothelial cells of the cerebellum is also defined as negative in diabetes type 2. The absence of a pronounced proliferation of vascular endothelium in diabetes type 2 is likely due to the toxic effects of excess amounts of glucose and insulin in a chronic state of insulin resistance, due to the peculiarities of glucose metabolism in the brain.

Expression of apoptosis marker p53 was defined as weakly positive in pericytes of arterioles and precapillaries cerebellum in diabetes type 2.

According to the computer morphometry the deformation of vessels is most expressed in the brain of patients with diabetes type 1 in the stage of lipohyalinosis. At this stage in patients with diabetes type 1 reduces the contractility and dilated possible of arterioles in the brain (according to the computer morphometry of small diameter arterioles have great indicators form factor r = -0,31 - inverse correlation average degree). Microvessels in diabetes type 2 have a larger diameter and the thickness of the vascular wall, which may be related not only to glucose toxicity, and toxicity to insulin, that is often observed in patients. Compensatory hyperinsulinemia in insulin resistance (depending on the degree of severity) are atherogenic factor due to increased vascular smooth muscle cell proliferation and extracellular matrix protein formation. Table 1 (indicators microvessel morphometry).

CONCLUSION

In the development of diabetic microangiopathy 3 stages can be identified: the reversible stage of impregnating vascular wall proteins and lipids of blood plasma, the stage of locally increasing the number of pericytes and vascular fibrosis, thickened wall, the irreversible stage of lipohyalinosis in the vascular wall.

Structural reorganization of walls of microvessels in the diabetes type 1 and type 2 is a long-term process and according to immunohistochemical study in the stage of fibrosis and hyalinosis characterized by low levels of expression of proliferation markers PCNA, Ki67 and p53 apoptosis in isolated endothelial cells and pericytes of cerebral vessels.

At the level of light microscopy pathological signs of microangiopathy in the diabetes type 1 and 2 do not differ significantly.

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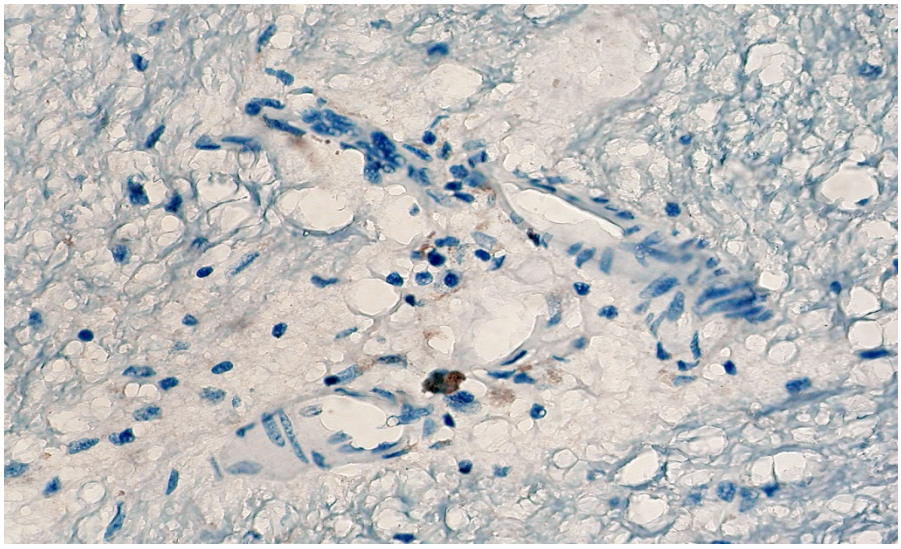

Fig.1. Expression PCNA pericytes of brain arteriolas at DM type 1

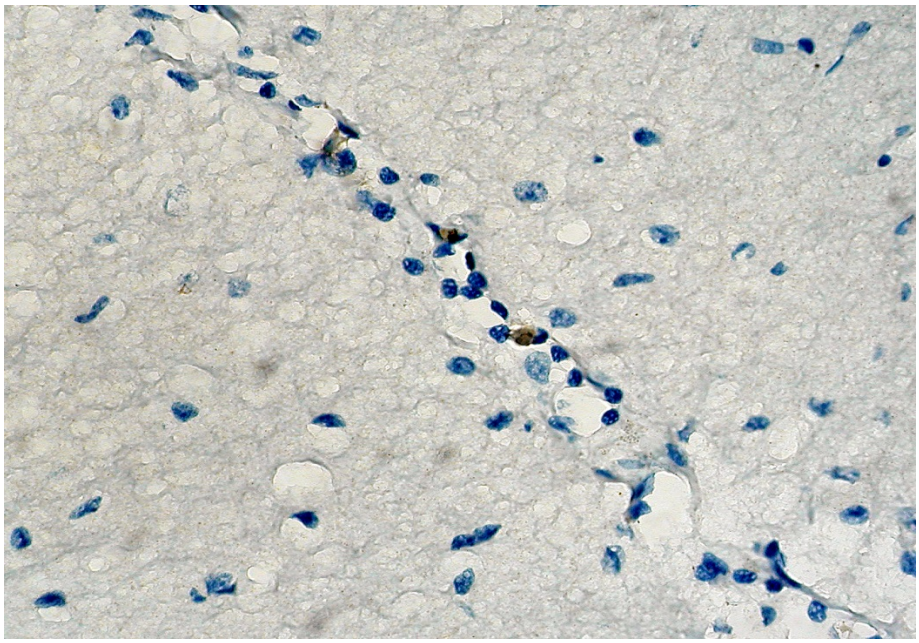

Fig. 2. Expression 3 in endothelia of brain precapillary at DM type 1

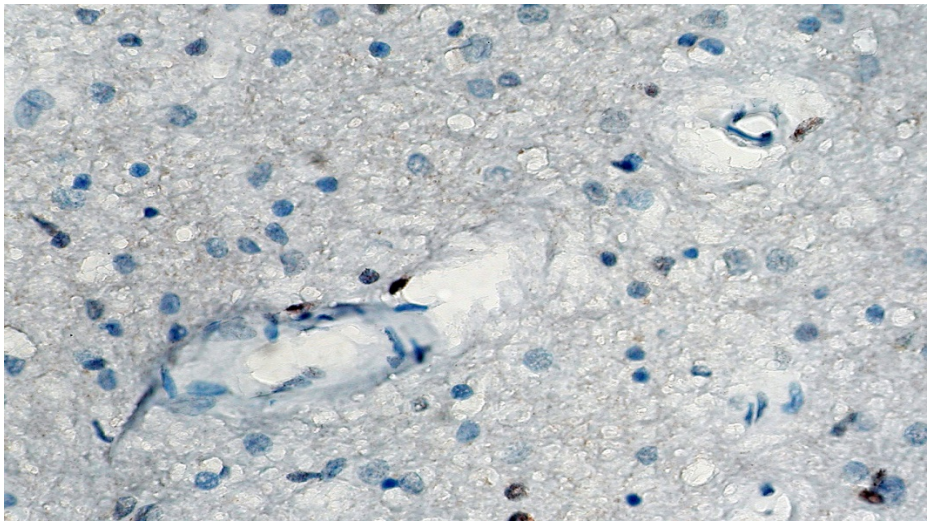

Fig. 3. Expression PCNA pericytes of brain arteriolas at DM type 2

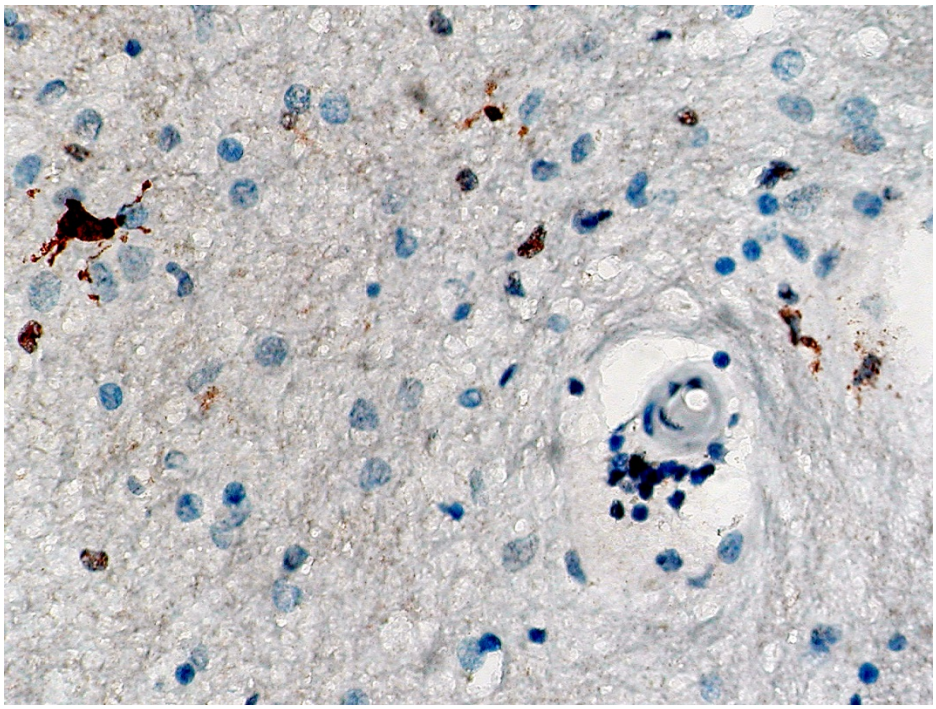

Fig. 4. Expression PCNA glial cells in the absence of expression of this proliferation marker Iin hyalinized precapillars of the brain type 2

Table 1

Showings of morphometry of microvessels

	Control (symptomatic hyperglycemia)	**DM Type 1**	**DM Type 2**
Brain			
1) diameter of vessels	22,75±1,86	26,28±2,45	29,668±3,03
2) value of form factor	0,89±0,012	0,77±0,028	0,83±0,02
3) thickness of vascular wall	3,44±0,16	6,30±0,37	7,43±0,40