CHOROIDAL VASCULARE CHANGES IN HYPERGLYCEMIC RATS EMBRYOS

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Summary

Diabetes is associated with an increased risk for cerebrovascular disease that happened in both microvascular and macrovascular complications.
In this research, the effect of maternal diabetes on the choroids plexus volume changes in different day of gestation in rat's embryos was studied.
Diabetes was induced by streptozotocin (55 mg kg^{-1}), given by a single intraperitoneal injection to female Wistar rats (200-250 g). Control animals were given an equivalent amount of citrate buffer saline. In six stage of neonatal life (16.5, 17.5, 18.5, 19.5, 20.5, 21.5dg), two embryo of each mother selected and under anesthetized the brain was removed and fixed. Serial cross –sections were cut and stained with hematoxylin and eosin. The volume of choroids plexus by stereological methods was measured.
Statistical analysis's showed significant difference in choroids plexus volume in all stage between diabetic and control groups ($p<0.01$).
This study shows that maternal diabetes causes microvascular disorders in choroids plexus system that lead to an increase in volumes of choroids plexus.

Key words: maternal diabetes, choroids plexus, embryo, microvascular disorders

Running title: Changes in choroids plexue volume

Introduction

Diabetes mellitus is an autoimmune disease characterized by destruction of insulin producing beta cells in the pancreas that over time may ultimately result in pathogenic diabetic complications(1). This disease is associated with an increased risk for cerebrovascular disease (2).Accumulating data support that oxidative stress induced by chronic hyperglaycemia plays a key role in both microvascular and macrovascular complications of diabetes, including stroke (3).
On the cardiovascular side, diabetes has long been associated with accelerated neuropathy(4).More recently, a number of abnormalities associated with disregulation of neovascularization have also been recognized. These include abnormally enhanced angiogenesis, defined as capillary vessel growth, in the retina, leading to diabetic retinopathy(5), in the vessel wall and cerebrovascular disorders (4).The significant morbidity and mortality of diabetes mellitus result predominantly from its complications, including blindness, renal failure(7), amputations, strokes and cardiac events(6).Maternal diabetes is associated with an increased risk of several complications in the offspring, such as growth disturbances and congenital malformations (8).

During vertebrate embryogenesis, the development of the vasculature of the head begins when angioblasts, which originate solely from the lateral splanchnis mesoderm, enter the head region and form the perineural vascular plexus by renovo formation of vessels, a process named vasculogensis(9)**.**Abnormal angiogenesis in diabetes is most clinically apparent in proliferative diabetic retinopathy(10). The neovascularization is preceded by the selective destruction of pericytes, capillary failure and hypoxia that leads to the release of pro-angiogenic substance (11).This study was aimed at investigating the changes in the choroids plexus capillaries(12) in the embryos from diabetic and normal mothers. For this aim we estimate the volume of choroids plexus in embryos from diabetic mothers to control in different day of gestation.

Material and methods

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2009). All chemical used in this study were purchased from Sigma (UK).

Experimental design:
Thirty six female Wistar rats were used for this study. The study was approved by the committee of our institute. Young adult female rats (approximately 250 gr) were maintained at 22c with 12-h periods of light and darkness. They were mated with normal males and the morning of appearance of vaginal plug was considered day 0 of gestation. At 7 days of gestation (dg), diabetes was induced by a single injection (i.p.) of streptozotocin (55mg/kg) dissolved in sterile phosphate buffered salin (13). Control group received only buffer. Then animals were housed under standard condition and received food and water. Induction of diabetes was confirmed by blood glucose level (glycemia > 400).
At the 16.5, 17.5, 18.5, 19.5 20.5, 21.5 days of gestation (dg), female pregnant rats were anesthetized under interaperitoneal injection of 0.24 cc of a mixture (1:2) of 10% ketamin and 2% xylazine and two embryo of each mother were selected. At the total for each day, There is six embryo of diabetic mother (n=6).

Groups
Thirty six female rats divided into two groups:
1) Control (n=18).For each day (16.5, 17.5, 18.5, 19.5 20.5, 21.5 dg) three mother were selected.
2) Experimental (n=18)

Blood assays:
Blood samples were collected from mothers at the three times (1. Two days after injection of STZ. 2. One week after injection of STZ. 3. At the day of surgery) and the levels of glucose by auto analyser were measured.

Sampling:
Under the pentobarbital anesthesia the embryos brain was rapidly removed and fixed in 10% paraformaldehyde .For histological evaluation samples were placed in same fixative over night and was embedded in paraffin. Serial cross –sections were cut and stained with hematoxylin and eosin(14).

Stereological methods:
The volume of choroid plexus was measured with Cavalieri method (15).
Cvalieri showed that the volume of any object may be estimated from parallel sections separated by a known distance ***t,*** by summing up the areas of all cross sections of the object ∑***a (prof***) and multiplying this figure by ***t:***

$$V(obj) = t. \sum a\ (prof)$$

Statistical analysis:
Student's t test was used for comparison when only two groups were analyzed and a one-way ANOVA followed by a Scheffe' f test when more than two groups were analyzed. Statistical significance was chosen as $p<0.05$. All results are reported as mean ± SEM.

Results

The results indicate several facts:

Blood and CSF chemistry

1. Diabetes were assessed in this study by monitoring the blood glucose levels in both PBS and STZ injected rats (Fig.1).There was a significant increase ($p<0.001$) in blood glucose levels from (100±5 mg.dl $^{-1}$) in control to (470±18 mg.dl $^{-1}$) in diabetic rats.

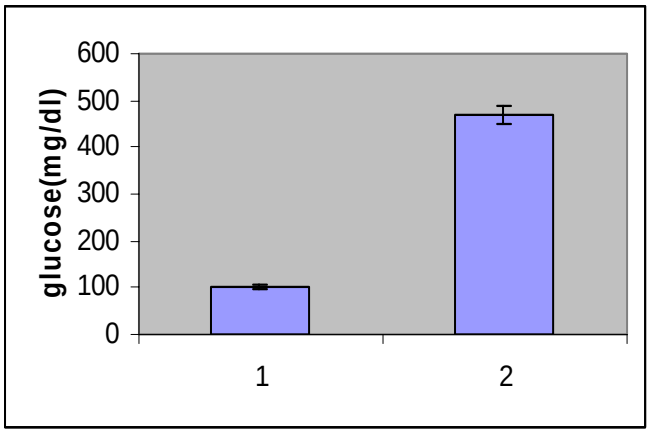

Fig.1: Comparing plasma glucose level (mg.dl $^{-1}$) in control and diabetic groups. (n=18). 1. Control; 2.Diabetic. (P<0.01)

Volume of choroids plexus

2. Results indicate that the volume of choroids plexus in embryos from diabetic mother was increased. The mean of choroids plexus volume in experimental group was (0.0945±0.0172) mm3 and in control group (0.0704±0.0130) mm3.This increase ($p<0.01$) was meaningful (Fig.2).

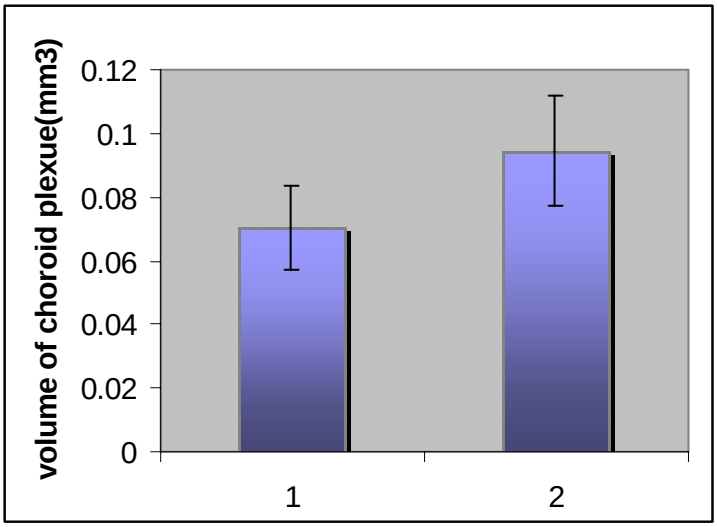

Fig.2. Comparing volume of choroids plexus in the embryo from diabetic (Experimental) and control rats in different days of gestation. (n=6). 1. Control; 2.Diabetic. (P<0.01).

3. Also in each day of gestation the volume of choroids plexus in embryos from diabetic mother was larger to control group in same day (Table.1).

Table.1: Comparing volume of choroids plexus in the embryo from diabetic (Experimental) and control rats in different days of gestation.

	Control	Experimental
Mean in 16.5 dg	0.033 ± 0.0009	0.042 ± 0.0004
Mean in 17.5 dg	0.0433 ± 0.0004	0.0618 ± 0.0053
Mean in 18.5 dg	0.06500 ± 0.001	0.08278 ± 0.004
Mean in 19.5 dg	0.0778 ± 0.005	0.0994 ± 0.004
Mean in 20.5 dg	0.08017 ± 0.0003	0.1215 ± 0.006
Mean in 21.5 dg	0.1229 ± 0.003	0.1593 ± 0.005
Mean ± SE	0.0704 ± 0.0130	0.0945 ± 0.0172

Values are means± SEM, n=6. p<0.01 indicates significant difference from control determined by Student s t test.

4. The size of choroids plexus has remarkable increased in embryos from diabetic mothers in compare with embryos from control mothers (Fig.3).

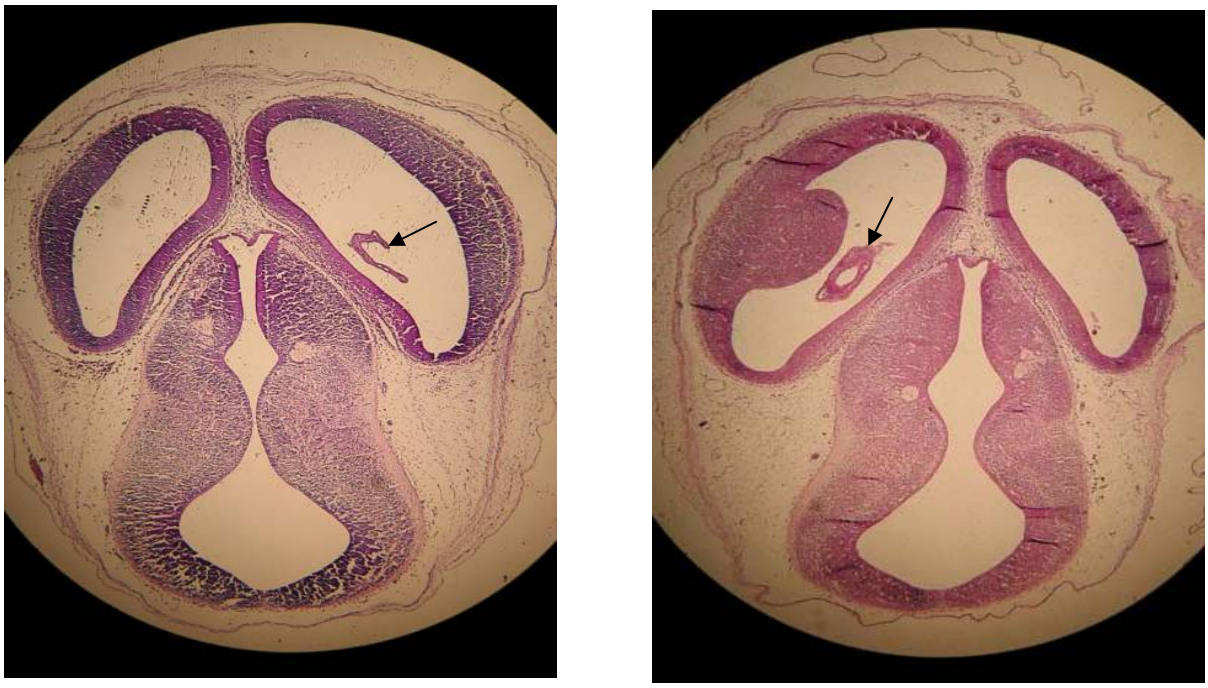

Fig.3. Cross section of embryo brain in different groups. haematoxylin-eosin. (x200)
Right panel: embryo from diabetic mother.
Left panel: embryo from control mother.
Choroids plexus was shown with arrows.

Discussion

Major findings in the present study were high risk for microvascular disease in embryos from diabetic mothers. Earlier studies have suggested that increased glucose lead to malformation. In addition, oxidative stress (3) disturbances in the polyol pathway and prostaglandin metabolism have been proposed to induce diabetic abnormally (6).

It has been suggested that enhanced activity of PKC may be a common feature of all diabetic complications (16). As diabetes progressed, it was evident that microvascular damage occurred even when hyperglycemia was controlled. From these studies, important factors, including prolonged hyperglycemia, hypertension increases oxidant stress, dyslipidemia and insulin resistance (17) have been shown to play a role in diabetes induced endothelial cell dysfunction. Few studies have investigated the effects of diabetes on the vasculature of the central nervous system (6). However, recent clinical evidence suggests diabetes leads to increased incidences of vascular dementia, ventricular hypertrophy, lacunar infracts and hemorrhage and may be a predisposing factor for Alzheimer's disease (18). Many studies measure BBB disruption by increased permeability of the microvasculature to albumin (19). We argue that by the time there is significant increase in total volume of choroids plexus in embryos from diabetic mothers to camper with control ($p<0.01$) (Fig.2).

Comparing the volume of choroids plexus in different days of gestation show that in all days (16.5, 17.5, 18.5, 19.5, 20.5, 21.5 dg), embryos from diabetic mothers have a significant increase in volume of choroids plexus (Table.1) that this increase is remarkable even in photo (Fig.3).May be the effects of maternal STZ-induced diabetes mellitus on embryo choroids plexus states, which are likely to result from the altered carbohydrate metabolism of their mothers (17).

Recent studies demonstrated that hyperglycemia is the metabolic hallmark of diabetes and leads to widespread cellular damage(20). Endothelial cells, which poorly regulate intracellular glucose, may be particularly vulnerable to hyperglycemia. Under appropriate physiologic and pathophysiologic conditions, growth factor (FGF), are released to initiate angiogenesis (21,22,23,24).

Further studies will be needed to evaluate the role of hyperglycemia in neonatal life and finding the alterations in microvascular system in embryos from diabetic mothers and focus on haw alterations in microvascular system related to the hyperglycemia condition in gestation period (25).

Experimental studies suggest that VEGF may stimulate the expression of adhesion molecules by endothelial cells and promote vascular inflammation (26). Intensive multi factorial intervention is associated with significantly improved cardiovascular outcome (27).

Our results suggest that, in the context of a hyperglycemia during developmental angiogenesis, the positive affect of diabetes on capillary density was overcome only by VEGF overexpression, where as responses to other vasoactive peptides were altered in the same conditions. In the evaluation of future trials testing pro-angiogenic factors in patients with diabetes, such interferences of hyperglycemia on the angiogenic response will have to be considered.

In total, it is concluded that maternal diabetes effect on brain microvascular system in embryonic time, especially choroids plexus.
Result showed that there is significant increase in total volume of choroids plexus in embryos from diabetic mothers to camper with control ($p<0.01$). Understanding the causes of diabetic vascular complications has become an increasingly important issue because of the rapid rising prevalence of diabetes.

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