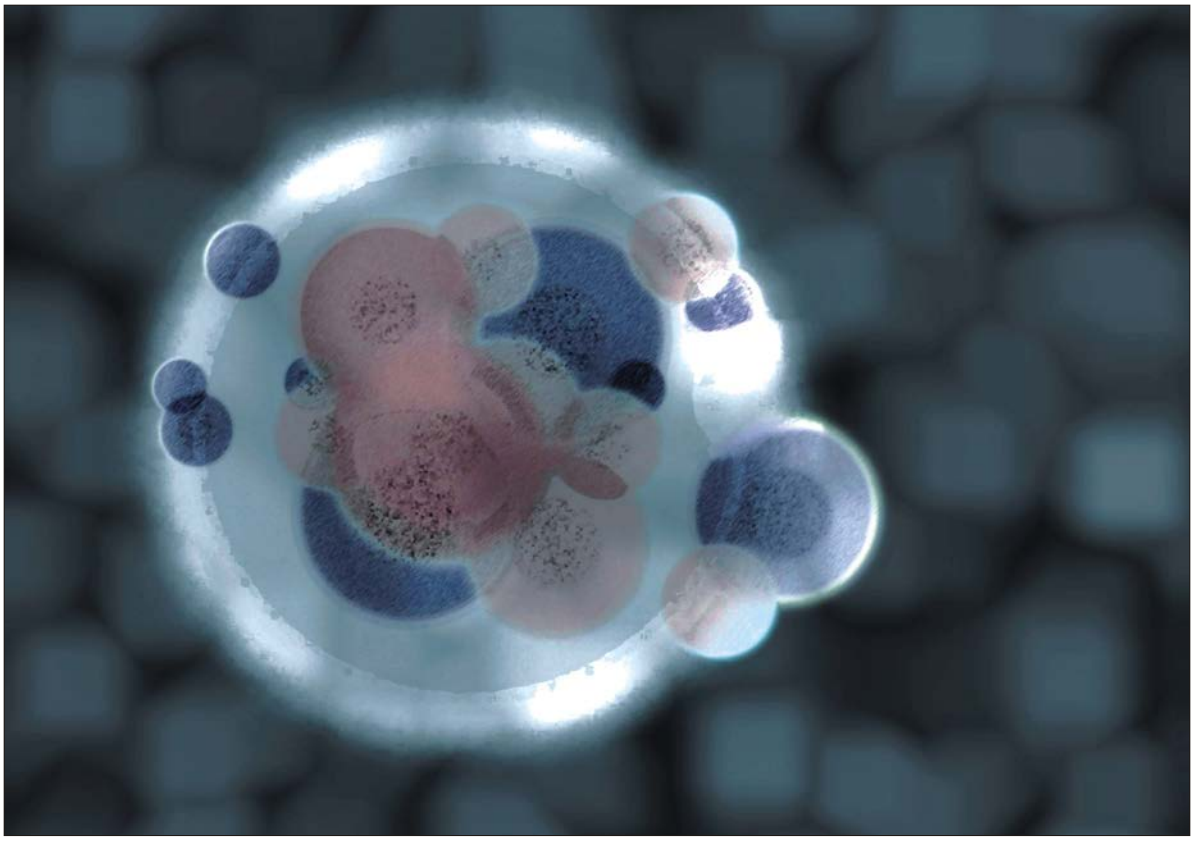

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Brain Ventricular Enlargement in Embryonic, Neonatal and Adulthood Stages of Rats Born from Diabetic Mothers

Tehranipour Maryam
Department of Biology, Faculty of Science, Islamic Azad University of Mashhad, Iran

Abstract: Structural brain abnormalities are more frequent in diabetes. Cerebrospinal fluid (CSF) is vital in controlling development of the nervous system along the whole length of the neural tube and the externalization of CSF during development is essential for the formation of the layers of neurons in the cerebral cortex. Previous studies have shown diabetes change CSF composition. In this study, we investigated the effect of maternal diabetes on lateral ventricle volume in developmental stages (embryonic, neonatal and adulthood). Diabetes was induced in female Wistar rats by intraperitoneal injection of STZ (55 mg kg^{-1} in citrate buffer, confirmed by glucose analysis: 100±5 mg dL^{-1} control, 470±18 mg dL^{-1} diabetic rats). At the 15, 1 and 30 day after birth, embryos and infants were anesthetized, brain were rapidly removed and fixed. Paraffin embedded tissue blocks were sectioned serially and stained with H.E. Then the volume of the lateral ventricle were measured with stereological methods. Results show in all stage of developing diabetic groups had significantly larger ventricle volume than controls ($p<0.01$). Therefore pregnant women who suffer from diabetes more likely to have a child with central nervous system problems, according to a new study.

Key words: CSF, lateral ventricle, hydrocephalus, diabetes, developmental

INTRODUCTION

Diabetes is a disease with numerous complications (Ristow, 2004). Though diabetes is a vascular disease (Spijkeman and Dekker, 2003), little is known regarding the effects of diabetes on the Blood Brain Barrier (BBB) or the Blood-cerebrospinal fluid Barrier (Malcolm, 2000). It has been reported that diabetes is a risk factor not only for stroke (Arvanitakis *et al*., 2004), but also for normal pressure hydrocephalus (Egleton *et al*., 2003). Normal pressure hydrocephalus occurs when the volume of CSF increases, but it is pressure remains normal or just slightly elevated (Flanagan, 2002). In addition, diabetes was associated with significantly reduced regional cerebral blood flow that hypoperfusion in the frontal region was significantly associated with gray matter atrophy and higher glycosylated hemoglobin was associated with significantly lower cerebral blood flow and greater within the temporal region (Last *et al*., 2007). Also diabetes increases blood brain barrier permeability. In either case more CSF flows into the ventricles (Flanagan, 2002). CSF is produced by the choroidal epithelial cells of the choroid plexus (Malcolm, 2000). Cerebrospinal fluid (CSF) has an important role in the developmental process (Wit *et al*., 2008). The fluid flow is essentially one-way and the location of the choroid plexuses in the lateral, third and fourth ventricles allows for the possibility of new components being added to the fluid at this points (Brown *et al*., 2004). Moreover, other sources of additions on the CSF exist, notably the sub-commissural organ, which sits at the opening of the third ventricle into the cerebral aqueduct and is the source of Reisners fibre, glycoproteins and unknown soluble proteins (Miyan *et al*., 2003). Several functions have been ascribed to the cerebrospinal fluid (CSF), including protection to the brain excretion of metabolites, homeostasis of the brain chemical environment, regulation of the correct chemical environment for neuronal function and as transport pathway between different brain areas (Tarnaris *et al*., 2006). These various functions, coupled to it is rapid turnover, perpetual formation and continuous circulation and absorption have led to consider the CSF as the third circulation (Pérez-Fígares *et al*., 2001). In the human, the bulk of the ventricular CSF is drained into the cisterna magna, from where it flows dorsally into the subarachnoid space of the cerebellum, caudally into the spinal subarachnoid space and rostrally into several basal cisterns of the brain stem and forebrain. From these cisterns, the CSF reaches the subarachnoid space of the cerebral hemispheres (Malcolm, 2000). About 70-80% of CSF is secreted by the choroid plexses that most of the choroidal CSF is secreted by the choroid plexuses of the

lateral ventricles. In higher vertebrates, the fetal cerebrospinal fluid is formed within the neural tube before the choroid plexus anlage appears (Brown *et al*., 2004). Studies in animal models and humans have shown that CSF contains essential molecules for neural proliferation and migration, which is predominantly secreted by choroid plexus and sub-commissural organ (Doublier *et al*., 2000). We propose that CSF is vital in controlling development of the nervous system along the whole length of the neural tube and that externalization of CSF during development is essential for the formation of the layers of neurons in the cerebral cortex (Miyan *et al*., 2003). Normal pressure hydrocephalus causes two problems for the brain. The first is that it increases the pressure acting on the structures that surround the ventricles. The second is that it stretches and enlarges the ventricles (Flanagan, 2002). This is important because when the ventricles become enlarged they stretch the white matter structures that are nearby.

The aim of present experimental design was to induce maternal diabetes mellitus and to assess the effects of that on lateral ventricle volume in embryonic, neonatal and adulthood stages of rat.

MATERIALS AND METHODS

All experiment were conducted in Faculty of Science, Islamic Azad University of Mashhad, Iran (2008). In this study Wistar rats (300-350 g) prepared from Razi Institute. Diabetes was induced in female rats at the 7 days of gestation (dg) via an intraperitoneal injection of 55 mg kg^{-1} STZ (streptozotocin) in sterile phosphate buffered saline (Calvo *et al*., 1997). Control animals were injected intraperitonealy with phosphate buffered saline. The animals were hosed under standard (12 h) light-dark conditions and received food and water. Induction of diabetes were assessed by blood glucose levels. The animals had above 400 mg dL^{-1} glucose levels were confirmed as diabetic animals.

Sampling: At the 15 day of gestation, 1 day after birth and 30 day after birth, we select embryo and infant of each diabetic and normal mother. All embryo and infants were anesthetized with sodium pentobarbital (64.8 mg kg^{-1}) and decapitated. The brain were rapidly removed and fixed in 10% paraformaldehyde. NaCl was added to the fixative to make the tissue float in order to ever come deformities during the fixation period. Paraffin embedded tissue blocks were sectioned at 7 mμ thickness coronally and stained with haematoxylin-eosin.

In addition, tissue blocks containing samples (brains) were serially cut throughout. Form several hundred sections per block, of each 20 section 3 serial section were obtained. For example for the first series: 21st, 22nd, 23rd section and for the second series: 44th, 45th, 46th section and so on. Therefore we mounted every 3 section on a slide. At a practical level, Stereological methods are precise tools for obtaining quantitative information about three-dimensional structures based mainly on observations made on sections. The volume of the lateral ventricle were measured with Cavalieri method (Gunderson *et al*., 1988). All experiments were performed a minimum of two times. Student's t-test was used for comparison when only 2 groups were analyzed. Statistical significance was chosen as $p<0.05$. All results are reported as mean±SEM.

RESULTS

Blood glucose levels: Glucose concentrations in the maternal plasma were as follows:

Control: 100±5 mg dL^{-1}, diabetic: 470±18 mg dL^{-1}. Glucose levels remained elevated throughout the study in diabetic mother in compare with control ($p<0.001$).

Lateral ventricles volume in embryo: There was a marked increase in the volume of ventricles in 15.5 embryos from diabetic mother in compare with control (Fig. 1). This increase was 0.49±0.04 mm^3 in control to 1.22±0.15 mm^3 in embryo from diabetic mother. Statistical analysis was shown a significant increase ($p<0.001$) in volume of lateral ventricles in embryo from diabetic mother (Fig. 2).

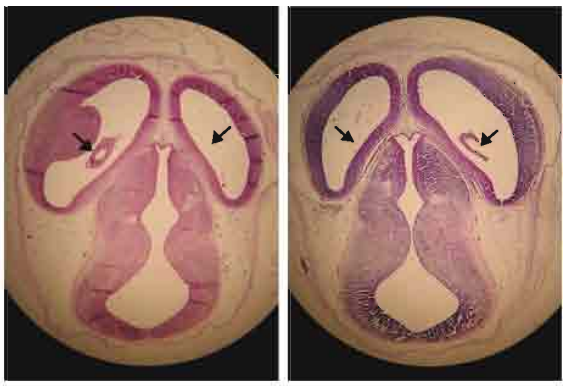

Fig. 1: Photomicrograph of the brain section of 15.5 dg. Right panel shows lateral ventricle in embryo from diabetic mother in comparison to the control left side (left panel) (X50). (arrows show lateral ventricle)

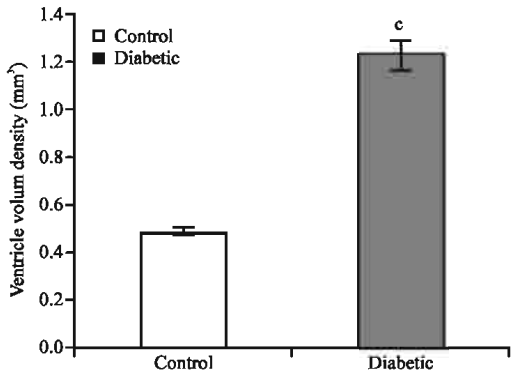

Fig. 2: Lateral ventricle volume in rat embryo 15 dg from diabetic and control mothers. Values are means±SD, n = 10; (c = p<0.001)

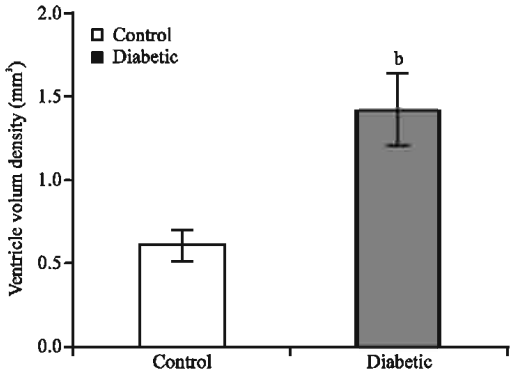

Fig. 3: Lateral ventricle volume in rat neonate from diabetic and control mothers. Values are means±SD, n = 10; (b = p<0.01)

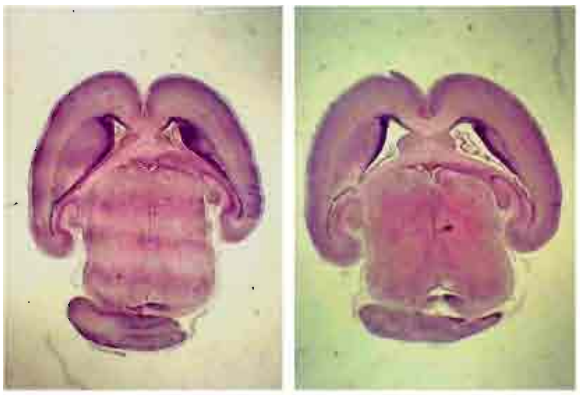

Fig. 4: Photomicrograph of the brain section of neonate. Right panel shows lateral ventricle in neonate from diabetic mothers in comparison to the control left side (left panel) (X3/3). (arrows show lateral ventricle)

Fig. 5: Photomicrograph of the brain section of 30 day-old rat. Right panel shows lateral ventricle in rat from diabetic mothers in comparison to the control left side (left panel) (X3/3). (arrows show lateral ventricle)

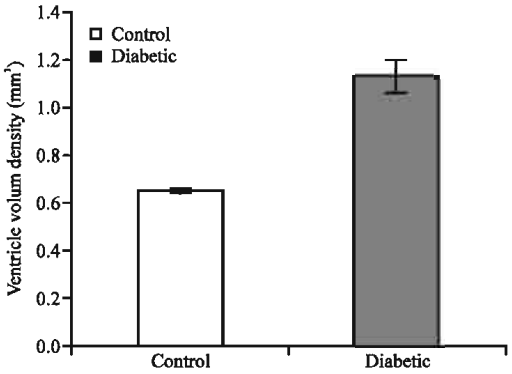

Fig. 6: Lateral ventricle volume in 30 day old rat from diabetic and control mothers. Values are means±SD, n = 10; (c = p<0.001)

Lateral ventricles volume in neonate (1 day old rat): Volume of lateral ventricles in neonate from diabetic mother was 1.41±0.20 to 0.614±0.09 mm³ in control (p<0.01) (Fig. 3). Increase in lateral ventricle in two groups (control and diabetic) was obviously observed in Fig. 4.

Lateral ventricles volume in (30 day old rat): There was a significant increase (p<0.001) in 30 day old rat from diabetic mothers as compared with controls (Fig. 5). The volume of Lateral ventricles in 30 day old rat from diabetic mother was 1.13±0.06 to 0.64±0.01 mm³ in control (Fig. 6).

DISCUSSION

Hydrocephalus is a net accumulation of CSF. Hydrocephalus results from an alteration of a normal

physiological process and has multiple causes with the rare exception of CSF overproduction from a choroid plexuses papilloma (Pérez-Fígares *et al*., 2001).

There is evidence that diabetes is a risk factor for normal-pressure hydrocephalus. Also Infants of diabetic mothers are exposured into high risk for hydrocephalus (Jarvis *et al*., 2005). NPH (normal-pressure hydrocephalus) is primarily associated with other diseases like AD and diabetes (Flanagan, 2002). Diabetes increases blood brain barrier permeability. In either case more CSF flows into the ventricles (Flanagan, 2002). Previous studies have shown diabetes change CSF composition (Nabiyouni *et al*., 2004). An evidence confirms that increase in BBB permeability for various ion transport in infant is resulted from STZ induced maternal diabetes (Tehranipour *et al*., 2007).

Hyperventilation and certain chemicals that were released in diabetic intra-uterne condition increase blood brain barrier permeability (Flanagan, 2002). Thus decreasing the CSF pressure gradient and CSF out flow this causes an increases in CSF volume. When the ventricles fill to capacity they start to compress the structures that are within and around them. When they become overfilled they begin to stretch. Eventually they become enlarged. At this study results show a meaningful increase in lateral ventricle volume in embryo ($p<0.001$) in 1-day-old ($p<0.01$) and 30-day-old rats ($p<0.001$) from diabetic mother in compared to control (Fig. 2, 3, 5).

Nonetheless, at a certain volume overfilling may affect structures on the surface of the brain stem such as the medulla, pons and lower cranial nerves. After the cisterns become overfilled, the ventricles start to fill up. In contrast to the basal cisterns, however the ventricles are surrounded by densely packed structures such as cerebellar, hypothalamic, thalamic and other important nuclei (Quarantelli *et al*., 2003). This limits their capacity to accommodate on increase in CSF volume compared to the cisterns and subarachnoid spaces and ventricles change shape under increased pressure such as were seen in Fig. 1, 4 and 6.

Although the mechanisms responsible for the CSF circulation are not fully understood, the following factors do play a role: 1) the hydrostatic difference between the production and drainage sites; 2) the pulsations the cerebral arterial tree; 3) the directional beating of ependymal cilia (Banks, 2006).

In mammals, the predominant route of escape of CSF into blood is through the arachnoid villi, with a small proportion escaping via the lymphatic system lying along nerve roots (Banks, 2006). The transcellular channels have a tortuous trajectory and this tortuosity might be responsible for a valvular effect, allowing the closure of the channel when the pressure in the blood vessel become too high (Egleton *et al*., 2003) as we saw in diabetic conditions.

In total, The diabetic group had significantly smaller global white and gray matter and larger lateral ventricle volumes than controls. It is concluded when an embryo growths in diabetic condition, this condition affect on cerebral development that this effect would be seen in all stages of development (embryonic, neonatal and adulthood). Maternal hyperglycemia induced CSF accommodation in brain ventricles and they become enlarged. Therefore the shape and size of ventricles and structures that surrounded them would change. CSF increasing and reduction of white and gray matter induced more central nervous system abnormality that resulted to some diseases such as those seen in schizophrenia, manic depression and AD that child from diabetic mother suffer from them. The researchers note that uncontrolled diabetes may contribute to such problems.

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