THE EFFECT OF MATERNAL DIABETES ON HYPOTHALAMUS NEURONAL CHEANGES IN NEONATE RATS

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Summary

Pregnant women who suffer from diabetes are more likely to have a child with central nervous system problems. In addition of vascular dysfunctions, maternal diabetes induces some changes in infant's brain structures. Hyperglycemia change expression of growth factors and differentiation in the developing embryonic brain.

The aim of present study has been undertaken the effects of maternal diabetes on hypothalamus neuronal density in neonate individual's rats from diabetic mothers in compare to control groups. Diabetes was induced by stereptozotocin (55mg kg^{-1}) given by a single intraperitoneal injection to female Wistar rats. Control rats were injected with phosphate buffered saline. In neonates brains rapidly were removed and in all sample the number of neurons in hypothalamus was measured via stereological method in both control and experimental groups. Statistical analysis determines that there is a meaningful reduction in number of neurons in neonate hypothalamus of diabetic mothers ($p<o.o5$). That may be oxidative stress increase tissue levels of highly reactive and toxic substances and effects signal transduction pathways involved in neuronal and endothelial cell function and induced neuronal degeneration in these neonates.

Key words: maternal diabetes, hypothalamus, neuronal density

Running title: maternal diabetes on hypothalamus

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Introduction

Autonomic and peripheral neuropathies are well-described complications in diabetes. Diabetes mellitus is also associated to central nervous system damage (1). This little-known complication is characterized by impairment of brain functions and electrophysiological changes associated with neurochemical and structural abnormalities (2,3). The pathogenesis of these deficits is multifactor and may involve microvascular dysfunction and oxidative stress (4).Cognitive deficits are also reported to occur in animal models of diabetes (Stroptozotocin induced)which can be prevented, but not fully reversed by insulin treatment (5).

Pathological changes in the central nervous system of diabetic animals and human are usually subtle in the brain(6) selective vulnerability was reported especially in the cerebral cortex and in the hypothalamus (the preoptic- suprachiasmatic nuclei) in diabetic rats(7).Previous study were established some ultrastructural changes in the hypothalamic paraventricular and supraoptic nucleus (8,9,10).

Light and electron microscope studies showed degenerative changes of neurons and glia, perivascular and mitochondrial swelling, disarrangement of myelin sheath, increased area of myelinated axons, presynaptic vesicle dispersion in swollen axonal boutoms, fragmentation of neurofilaments, and oligodendrocyte abnormalities. In addition, depressive mood was observed in diabetic animals. The brain morphological alterations observed in diabetic animals could be related to brain pathologic process leading to abnormal function, cellular death, and depressive behavioral (11).

Pregnant women who suffer from diabetes are more likely to have a child with central nervous system problems; according to a new study. Fetal brain iron deficiency occurs in human pregnancies complicated by diabetes mellitus or more common in the offspring of these pregnancies. The aim of present experimental design was to induce maternal diabetes mellitus and to assess the effects of that on the hypothalamus neuronal density.

Materials and Methods

Female Wistar rats, weighting 200-250g, were hosed under standard laboratory conditions and kept under natural 12h light: 12h dark cycle. The animals procured

from Razi Animal House, were housed 2 per cage with free access to standard food and water. Rats were acclimatized to laboratory conditions before testing. Animal were divided into 2 different groups for estiminat the effect of maternal diabetes in neonates.

Group 1- Normal- rats were not subjected to any procedures (n=8) .

Group 2- Diabetes- under STZ injection (n=8).

All the experimental protocols were conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2010).All chemical used in this study were purchased from Sigma (UK).

Induction of diabetes:

Diabetes was induced in rats by a single injection of STZ ($55mgkg^{-1}$) (12) freshly dissolved in citrate buffer(PH 4.5).Age –matched control animals were injected with citrate buffer. Diabetes was confirmed after 8 weeks; only the animals with blood glucose level above 400 mg/dl were included in the study.

The body weight was measured at the beginning and the end of the experiment. All animals were checked for glucose blood concentration at the beginning of the experiment. After 2 weeks from STZ-injection, they were as well as on the day before of experiment.

Tissue collection:

After the bear the pups, one pup from each mother was selected randomly. Neonates were anesthetized with sodium pentobarbital (64mg/kg) and decapitated. The whole brain was removed and fixed in 10% paraformaldehyde. NaCl was added to the fixative to make the tissue float in order to overcome deformities during the fixation period paraffin embedded tissue blocks were sectioned at 7mμ thickness coronally and stained with haematoxylin-eosin(13).

Measurement of neuronal density in hippocampus

Hemotoxylin-eosin-stained serial paraffin sections were prepared from 8 hypothalamus from individual animals in each group. Regions of hypothalamus were identified according to paxinos and Watson(14). Tissue blocks containing samples (brains) were serially cut throughout. Form several hundred sections per block, of each 20 section 3 serial sections were obtained. For example for the first series: 24^{st}, 25^{st} , 26^{st} section and for the second series :46^{st} ,47^{st} ,48^{st} 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 hypothalamus was measured with Cavalieri method and the neuronal density by dissector method as described by (15).

Statistical analysis:

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 ± SD.

Results

1-Two weeks after STZ-injection the rat's blood glucose level was higher than 400mg/dl.

2-The body weight of diabetic animals increased to(230-260 g) during the experimental time, whole in the normal to (270-340 g).

3-In the urine, all diabetic rats demonstrated abnormal results: glucosuria (+++), ketone bodies (trace), and protein (trace).In the plasma all diabetic rats have had remarkable increase in Creatinine, Uric acid, Urea, Triglycerides, Cholesterol, [P^{3+}], [Ca^{2+}] ($P<0.05$).

4-Maternal hyperglycemia produced evoked significant neuronal less in hypothalamus of neonate brains (Fig.1)This decrease was(60000±966) in control to (45417±300) in neonates from diabetic mothers(p<o.o5).

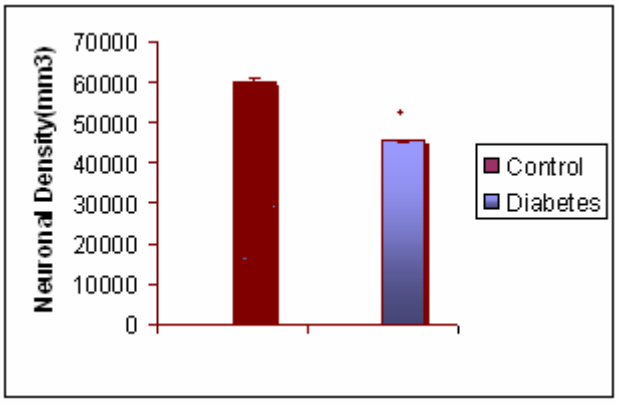

Fig.1: Neuronal density of hypothalamus in neonate from diabetic mothers compare to control.The values are presented as means± SEM. n=8. *P<0.05 Student s t test compare pups from Diabetic dams with pups from controls.

5- The volume of hypothalamus in neonate from control mother is (4475 ±2) to compare with neonate from diabetic mothers (4430±7) that has had a meaningful decrees (p<o.o5) (Fig.2).

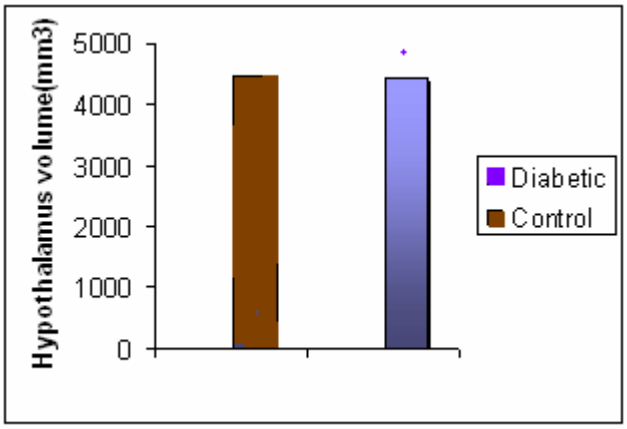

Fig.2: hypothalamus volume in neonate from diabetic mothers compare to control. The values are presented as means± SEM. n=8. *P<0.05 Student s t test compare pups from Diabetic dams with pups from controls.

6-In morphometric study, there was not any change in hypothalamus shape in neonates from diabetic mothers camper to controls (Fig.3).

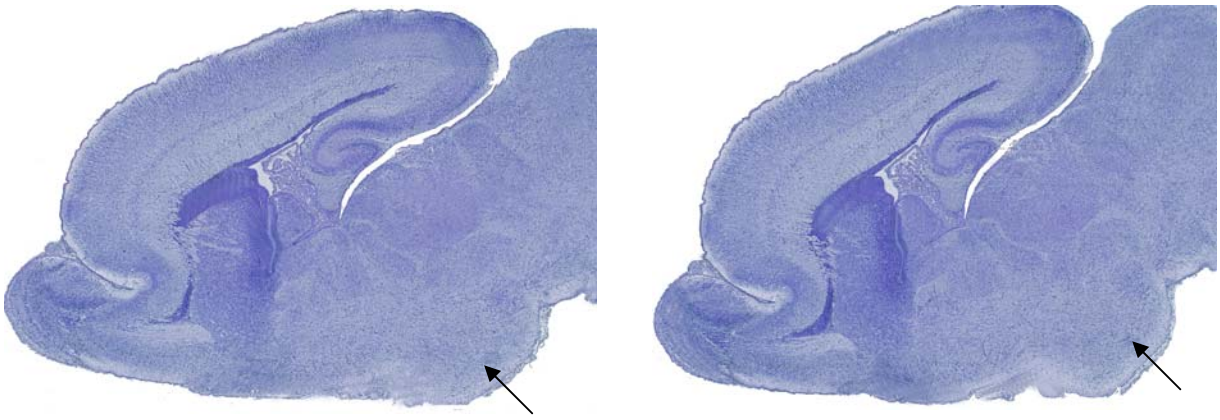

Fig.3: Photomicrograph of the brain section of neonate. Right panel: hypothalamus of neonate from diabetic mother. Left panel: Control. (X20).

Discussion

In our morphometric studies on hypothalamus neuronal density, it has been shown that the experimental maternal diabetes in rat's results neuronal loss and damage (Fig1), also the hypothalamus volume in experimental groups has had remarkable decrees (Fig2).

Previous study was shown some ultrastructural changes in the hypothalamic paraventricular and supraoptic nucleus. The hyperosmolality associated with diabetes mellitus triggers an increase in neuronal activity and vasopressin production within magnocellular neurosecretory cells (MNCs) of the hypothalamic supraoptic nucleus (SON)(8).

Although upregulation of vasopressin production in response to acute hyperosmolality is adaptive, prolonged overstimulation of vasopressin-producing neurons in chronic diabetes results in neurodegeneration and apoptosis (8). The causative factors for these changes are not clear. However, it is suggested that the osmotic stress caused by chronic dehydration in the diabetic animals may be partly or wholly responsible for these ultrastructural changes (9).

Diabetes causes morphometric neuronal changes (16). This observation has allowed us to postulate that the neuronal death in the infant from diabetic mothers proceeds on an apoptotic pathway (17). Gestational conditions increase fetal iron demand for erythropoiesis beyond placental iron transport capacity .Diabetes mellitus can result in a 30-40% reduction in neonatal brain iron (18).Iron in the form of cytochoromes is a required component of cellular oxidative metabolism in the brain and is thus essential for normal neuronal function(19).Metabolic diseases such as diabetes and obesity have been associated with increased vulnerability to stress (18)

Oxidative stress induced by chronic hyperglycemia contributes to cerebrovascular complication in diabetes(18).Also diabetes mellitus is associated with an increased risk for cerebrovascular disease(20).Accumulating data support the conclusion that oxidative stress induced by choronic hyperglycemia plays a key role in both microvascular and macrovascular complications of diabetes, including stroke(21).Many deleterious events contribute to oxidative damage to neurons in diabetes: because of high levels of polyunsaturated lipids in the brain, direct lipperoxidation frequently occurs causing lipid membrane disruption and consequent neurodegeneration (22).

Moreover, oxidative stress increase tissue levels of highly reactive and toxic substances and effects signal transduction pathways involved in neuronal and endothelial cell function. These results and our results confirm that an oxidative imbalance occurs in the hypothalamus of neonates born from diabetic rats as we have previously shown in diabetic rats. These data suggest that antioxidants effect some steps of signaling events leading to phosphorylation, ubiquination and degradation (4).The role of oxidative stress and Nk-kB activation on diabetic complications is well documented, moreover antioxidant treatment exerts a beneficial effect in experimental models of chronic injury in diabetes and treatment with antioxidants can significantly reduce diabetic complications(23).Reactive oxygen species activates a variety of target genes linked to the development of diabetic complications (24).

In addition, the loss of arachidonic acid content of the synaptosomal membrane, induced by diabetes and by transient cerebral ischemia, making the membrane more resistant to oxidative stress. Oxidative stress induced by chronic hyperglycemia directly can damage ionic homeostasis and membrane transport systems in the brain (25) and may be this is one of the reasons for hypothalamus neurons death. Apoptosis in diabetes has been ascribed to hyperglycemia and oxidative stress (26).

The other reason for neuronal death in diabetic pregnancy is Ischemia. It has been postulated that neurons with higher oxygen consumption in normal conditions are more susceptible to ischemia insult (22). It has been suggested that diabetes and ischaemia evoke the oxidative stress following an impairment of the respiratory chain in mitochondria and an overproduction of the reactive oxygen species (ROS). ROS are considered as a main factor in the pathogenesis on neuronal death 27).

Pathomechanism of degenerative changes and neuronal loss through apoptosis or necrosis is not clear until now. It has been suggested that changes in intracellular calcium concentrations in oxidative stress may indicate the pathway of cell death .It is suggested that more sever injury with high intracellular calcium concentration(Ca^{+2}) promotes necrotic cell death, where low (Ca^{+2}) and milder injury promotes cell death through apoptosis(28). Studies on antioxidative treatment would deliver further data important in the exploration of neuronal death in diabetes and ischemia.

In total, it is concluded that maternal diabetes induces some changes in hypothalamus neuronal structure and density. Statistical analysis show significant decrease in neuronal density (ND) and volume of hypothalamus in neonates from diabetic

mothers compare to control .Therefore, it is better in diabetic pregnant mother the level of glucose was maintained under normal condition.

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