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Alterations of the Giant Pyramidal Neurons (Betz Cells) in Brain Cortex of Rat Offspring Born from Gestational Diabetic Dams: A Morphometric Study

Alteraciones de las Neuronas Piramidales Gigantes de la Corteza Cerebral en Crías de Ratas Nacidas de Hembras con Diabetes Gestacional: Un Estudio Morfométrico

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SUMMARY: A few studies reported the adverse effects of gestational diabetes on hippocampus and spinal cord of rat offspring. Giant pyramidal neurons are giant pyramidal neurons located in fifth layers of the gray matter in the primary motor cortex. Therefore, this study was conducted to determine the effect of gestational diabetes on the giant pyramidal neurons and the thickness of internal pyramidal layer in the brain cortex of rat offspring. On day 1 of gestation, 10 Wistar rat dams were randomly allocated into two control and diabetic groups. Five animals in diabetic group received 40 mg/kg/BW of Streptozotocin (intraperitoneally) and control animals received normal saline. We randomly selected six offspring of every subject in both groups at day 28, 56 and 84. Rat offspring were sacrificed and then coronal sections were taken from the right brain cortex and sections were stained with Cresyl violet. The density of giant pyramidal neurons in brain cortex and thickness of internal pyramidal layer of brain cortex were evaluated. In P28, P56, P84 the Betz cells density of brain cortex were significantly reduced from 107.6 ± 6.2 , 131.6 ± 4.6 and 143.5 ± 4.0 in controls to 84.96 ± 2.1 , 109.8 ± 7.3 and 121.05 ± 5.6 in cases ($p < 0.05$), respectively. The thickness of the internal pyramidal layer of brain cortex in P28, 56 and P84 was significantly higher in gestational diabetic group in comparison with the control group ($p < 0.05$). This study showed that uncontrolled gestational diabetes reduces the giant pyramidal neurons density and internal pyramidal layer thickness in brain cortex of rat offspring.

KEY WORDS: Gestational diabetes; Neuron; Giant pyramidal neurons; Cerebral cortex; Rat offspring.

INTRODUCTION

Diabetes mellitus as a serious metabolic disorder characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins (Lebed *et al.*, 2008) and is associated with cerebral alterations (Biessels *et al.*, 2002; Gispen & Biessels, 2000; McCall, 1992) including abnormal expression of hypothalamic neuropeptidase, hippocampal astrogliosis (Saravia *et al.*, 2002) decreased hippocampal synaptic plasticity, neurotoxicity, changes in glutamate neurotransmission (Gardoni *et al.*, 2002) and hippocampal neuron density are dysfunctions of the central nervous system (Golalipour *et al.*, 2012).

Diabetes mellitus classified to type I or insulin dependent, type II or insulin independent and Gestational diabetes (Persaud, 2007). Gestational diabetes mellitus (GDM) defined as impaired glucose tolerance affects

approximately 4% of all pregnant women who have never before had diabetes, but who do have high blood glucose levels during pregnancy (Persaud).

Several studies have reported the adverse effects of diabetic pregnancy on the developing brain including neurobehavioral deficits in both sensory-cognitive and psychomotor functions, such as altered auditory recognition memory processing at birth (Siddappa *et al.*, 2004), reduced visual and memory performance at 8 and 12 months (DeBoer *et al.*, 2005), poorer performance on tests of general development in infants and toddlers and inferior performance in elementary school children (Ornoy, 2005), motor delay may be a sign of mild, nonspecific brain damage and the abnormalities in memory processing (Nelson *et al.*, 2000).

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Although, several studies reported the adverse effects of type I and type II diabetes mellitus on hippocampus, hypothalamus, cerebellum and cerebrum (Ahmadpour & Haghiri, 2011; Beauquis *et al.*, 2006; Jackson-Guilford *et al.*, 2000; Khaksar *et al.*, 2010; Li *et al.*, 2002; Piotrowski *et al.*, 2001) and studies have shown the adverse effects of gestational diabetes on hippocampus, spinal cord and cerebellum (Golalipour *et al.*, 2012, 2014; Najafdari *et al.*, 2014; Razi *et al.*, 2014), but there is no study about the effect of gestational diabetes on neuronal development of the giant pyramidal neurons and internal pyramidal layer thickness in brain cortex. Therefore, this experimental study was designed to assess the effect of gestational diabetes on the giant pyramidal neurons density and internal pyramidal layer thickness in brain cortex of rat offspring in the postnatal 28, 56 and 84 days of Wistar rats.

MATERIAL AND METHOD

This experimental study was performed at the Golestan University of medical sciences, Gorgan, Iran. Guidelines on the care and use of laboratory animals and approval of the ethic committee of Golestan University of medical sciences were obtained before study.

Experimental animals. Wistar rats, weighing 180–220 g (12 weeks old) were used in this study. The animals were maintained in a climate-controlled room under a 12-hour alternating light/dark cycle, 20 °C to 22 °C temperature, and 50% to 55% relative humidity. Dry food pellets and water were provided ad libitum.

Drug . Streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in sterile saline solution (0.85%) to give 40 mg/kg dose intraperitoneally (IP) injected to female rats.

Animal groups and treatment. After 2 weeks of acclimation to the diet and the environment, female Wistar rats were placed with a proven breeder male overnight for breeding. Vaginal smears were done the next morning to check for the presence of sperm. Once sperm was detected that day was assigned as gestational day 1(GD). On day 1 of gestation, pregnant females randomly divided into two control and diabetic groups (Fahrenkrog *et al.*, 2004).

Five female rats in diabetic group receiving 40 mg/kg/body weight of streptozotocin (STZ) and control groups (five rats) receiving an equivalent volume normal saline injection intraperitoneally. Blood was sampled from the tail at 1 week after STZ injection. The mothers with blood glucose level 120–250 mg/dl known as gestational diabetic mothers (Schoenle *et al.*, 2002).

The pregnancy of dams was terminated physiologically. In postnatal days of 28, 56 and 84, from each dam in control and cases six offspring randomly selected and were scarified. For light microscope preparations brain was fixed in 10% neutral-buffered formalin for histological procedure. The coronal sections (6 micrometer) serially collected from bregma -3.12 mm to 4.68 mm of the primary motor cortex (Paxinos & Watson, 1998). The sections were stained with cresyl violet.

Blood glucose measurements . Blood glucose level of mothers (before mating and after STZ injection) and offspring was obtained via tail vein and was estimated with a glucometer (ACCU-CHEK® Active Glucometer, Roche Diagnostics, Mannheim, Germany).

Morphometric techniques. For histomorphometric study, the sections were observed under the light microscope. In each offspring, ten similar sections of anterior to posterior of the primary motor cortex were selected and images by Olympus BX 51 microscope and DP12 digital camera

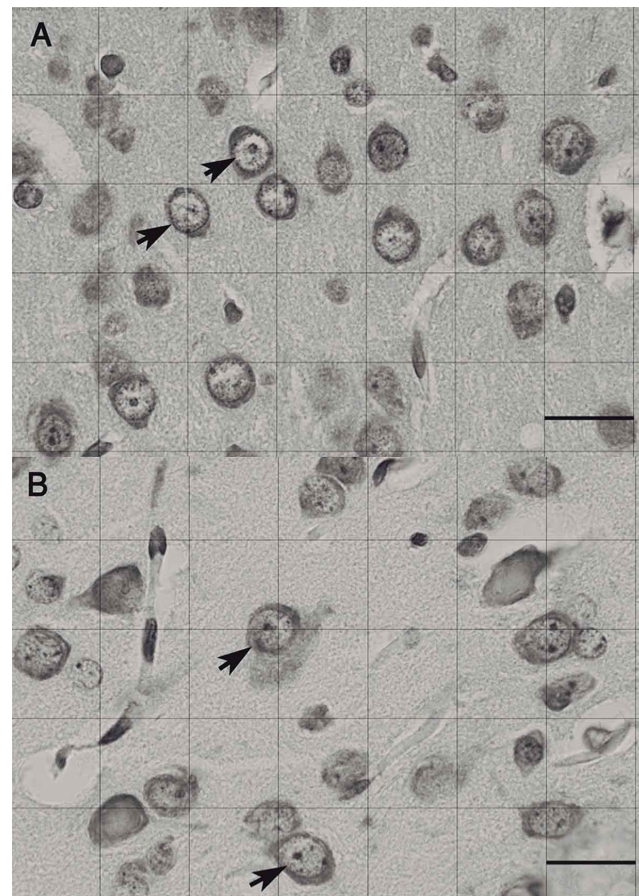


Fig. 1. Histological photograph of brain cortex, primary motor area in Wistar rat offsprings (P32) in control (A) and case (B) groups (1000X magnification, Grid: 20x20 μm , Scale bar: 20 μm , Cresylviolet staining, Arrows showing the pyramidal cells in IPL).

attached to OLYSIA autobioreport software (Olympus Optical, Co. LTD, Tokyo, Japan). The density of the giant pyramidal neurons was evaluated in 100000 μm^2 area of primary motor cortex in 1000X magnification (Fig. 1). Also the thickness of the Betz layer was obtained from 200X magnification.

Statistical analysis. Morphometric data are summarized with the Mean \pm SE. the results were analyzed with the Student's "t" test using SPSS 16 software. P<0.05 was considered significant.

RESULTS

The morphometric findings are depicted in Table I. The density of the giant pyramidal neurons in 100000 μm^2 area of primary motor cortex in P28, significantly reduced from 86.85 \pm 2.1 in controls to 64.14 \pm 4.2 in gestational diabetics offspring (p<0.008).

Also, the density of the giant pyramidal neurons in primary motor cortex of treated group significantly reduced from 74.85 \pm 3.0 to 60.42 \pm 4.0 in P56 and from 65.25 \pm 2.2 to 49.57 \pm 2.5 in 10000 μm^2 area in P84 (p <0.01).

The area and diameter of the giant pyramidal neurons, in primary motor cortex in P28, P56 and P84 in the treated groups were significantly increased in comparison with the controls (Table I).

The mean thickness (μm) of the Betz layer in the primary motor cortex in P28, P56 and P84, significantly increased in diabetics (944.63 \pm 26.0, 917.62 \pm 43.4,

1071.31 \pm 25.5) in comparison with the controls (755.5 \pm 29.0, 810.88 \pm 20.2, 972.46 \pm 26.1) (p<0.05).

DISCUSSION

The present study demonstrated that gestational diabetes produces a significant reduction in the giant pyramidal neurons density of brain cortex of rat offspring in the postnatal days of 24, 56 and 84.

Previous animal model studies have shown that dams with type 1 and 2 diabetes mellitus born offspring with low neuronal density in hippocampus (Beauquis *et al.*, 2006; Li *et al.*, 2002), catecholaminergic systems of hypothalamus (Plagemann *et al.*, 1998), granule layer of dentate gyrus (Ahmadpour & Haghiri) and cerebrum (Khaksar *et al.*).

Also, several studies have reported reduction of neuronal density in dentate gyrus of animals with type 1 and 2 diabetes mellitus (Hwang *et al.*, 2008; Kim *et al.*, 2003; Li *et al.*, 2002).

Hwang study showed that type II diabetes reduced hippocampal cell differentiation in the subgranular zone of the dentate gyrus in rat model (Hwang *et al.*).

Furthermore, Khaksar *et al.* study, showed that neural cell density in gray matter of brain and spinal cord reduces in offspring of diabetic rats. Martinez-Tellez *et al.* (2005), explained that diabetes mellitus might affect on the dendritic morphology in the limbic structures, prefrontal cortex, occipital cortex, and hippocampus.

Table I. The quantitative characteristics of the Betz cells of cerebrum and internal pyramidal layer thickness in postnatal day 28, 56 and 84 of gestational diabetes mellitus (GD) and control mothers in Wistar rat.

	Characteristics	Control	GD	P- value
P28	Betz density (Betz Number /100000 μm^2 area of IPL)	86.85 \pm 2.1	64.14 \pm 4.2	0.008
	Area of Betzs (μm^2)	109.34 \pm 6.6	153.56 \pm 7.2	0.03
	Diameter of Betzs (μm)	12.30 \pm 0.2	13.24 \pm 0.3	0.001
	Thickness of IPL (μm)	755.5 \pm 29.0	944.63 \pm 26.0	0.005
P56	Betz density (Betz Number /10000 μm^2 area of IPL)	74.85 \pm 3.0	60.42 \pm 4.0	0.01
	Area of Betzs (μm^2)	105.95 \pm 3.3	172.39 \pm 6.8	0.001
	Diameter of Betzs (μm)	10.06 \pm 0.2	12.5 \pm 0.4	0.001
	Thickness of IPL (μm)	810.88 \pm 20.2	917.62 \pm 43.4	0.04
P84	Betz density (Betz Number /10000 μm^2 area of IPL)	65.25 \pm 2.2	49.57 \pm 2.5	0.001
	Area of Betzs (μm^2)	147.29 \pm 4.4	181.38 \pm 10.1	0.001
	Diameter of Betzs (μm)	12.63 \pm 0.2	13.54 \pm 0.2	0.02
	Thickness of IPL (μm)	972.46 \pm 26.1	1071.31 \pm 25.5	0.02

Results are expressed as Mean \pm SE of the mean, n= 6, IPL= internal pyramidal layer.

Also, our previous studies indicated that gestational diabetes reduces the neuronal density of CA1 and CA3 of hippocampus, anterior horn of spinal cord and cerebellum cortex in rat offspring (Golalipour *et al.*, 2012, 2014; Najafdari *et al.*; Razi *et al.*).

In spite of several studies regarding the effects of diabetes I and II and gestational diabetes on CNS, there is no investigation about the effect of gestational diabetes on brain cortex neurons in offspring.

Our animal model study demonstrated that gestational diabetes similar to type I and II diabetes mellitus, has a neurotoxic effect on offspring brain cortex neurons.

Regardless of diabetes mellitus type, it is associated with hyperglycemia. Several possible mechanisms are explained about cerebral alterations including neuronal loss due to hyperglycemia.

One possible mechanism in cause of program cell deaths in diabetes mellitus (Allen *et al.*, 2005; Arroba *et al.*, 2003, 2005, 2007; Klein *et al.*, 2004; Lechuga-Sancho *et al.*, 2006a, 2006b; Nishikawa & Araki, 2007) can be due to decrease of insulin or insulin-like growth factor signaling (Ishii, 1995) or an increase in cytokines such as TNF α (Chen & Goeddel, 2002).

Moreover, insulin-like growth factor has a neuroprotective anti-apoptotic effect (Rizzo *et al.*, 1991) and down regulation of expression of insulin-like growth factor and its receptor in diabetes might also be expected to lead to neuronal loss (Li *et al.*, 2002; Russell & Feldman, 1999).

Diabetes mellitus is considered a chronic endogenous stressor which is related with increased oxidative stress in central nervous system (Ahmadpour *et al.*, 2010; Grillo *et al.*, 2005).

CNS complications of diabetes mellitus could be mediated through excessive free radicals generation (Ahmadpour & Haghiri; Okouchi *et al.*, 2005; Ziegler *et al.*, 2004). Free radicals by oxidizing proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes contribute to increased neuronal death (Hawkins & Davies, 2001).

Indeed, several studies have shown that offspring of diabetic mothers have lower arachidonic acid (AA:20:4n-6) and docosahexaenoic acid (DHA:22:6n-3) in cord blood (Min *et al.*, 2005; Wijendran *et al.*, 2000). Arachidonic acid metabolite and prostaglandin E2 play an important role in neurogenesis (Uchida *et al.*, 2002).

Zhao *et al.* (2009) have reported that maternal arachidonic acid supplementation improves neurodevelopment in young adult offspring from rat dams with and without diabetes. Other factors in active response in hyperglycemia, is down regulation of nitric oxide synthase (NOS) mRNA and protein concentrations are within hippocampal CA1 and CA3 neurons (Reagan & McEwen, 2002).

This down regulation of NOS mRNA may provide a partial explanation for the impaired long-term potentiation that is seen in the diabetic hippocampus, because induction and maintenance of potentiation are dependent on NOS activity and experimental inhibition of NOS decreases long-term potentiation and impairs cognitive learning and memory (Hölscher, 1997).

Diabetes may enhance the development of stroke via increased cortical apoptotic activity but this was not additive in the hippocampus following ischemic injury (Li *et al.*, 2004).

Also, the thickness of internal pyramidal layer in primary motor cortex may be due to increasing microglia and other glial cells because of inflammation reaction and increased vascular permeability in brain vessels and nervous cell death and astroglial reaction following realize cytokine and interleukin 1 (Beauquis *et al.*, 2008).

This study showed that uncontrolled gestational diabetes induces adverse effects on Betz neurons in offspring brain cortex, which remained persistent during postnatal period. Further studies are required for exploring the exact mechanism of CNS complications of gestational diabetes mellitus.

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GHAFARI, S.; GOLALIPOUR, E. & GOLALIPOUR, M. J. Alteraciones de las neuronas piramidales gigantes de la corteza cerebral en crías de ratas nacidas de hembras con diabetes gestacional: un estudio morfométrico. *Int. J. Morphol.*, 33(3):1120-1125, 2015.

RESUMEN: Pocos estudios han informado de los efectos adversos de la diabetes gestacional sobre las células del hipocampo y médula espinal. Este estudio, se realizó para determinar el efecto de la diabetes gestacional sobre las neuronas piramidales gigantes ubicadas en la quinta capas de la sustancia gris en la corteza motora primaria y el espesor de la capa piramidal interna en la corteza cerebral en crías de ratas. En el día 1 de la gestación, 10 ratas Wistar se asignaron aleatoriamente en dos grupos: control y diabéticos. Cinco animales del grupo diabético, fueron inyectados con 40 mg/kg de peso corporal de estreptozotocina (por vía intraperitoneal), y los de el grupo control, con solución salina. Aleatoriamente, se selecciona-

ron seis crías de cada hembra de ambos grupos los días 28, 56 y 84. Las crías fueron sacrificadas, se tomaron secciones coronales de la corteza cerebral derecha y se tiñeron con violeta de cresilo. Se evaluó la densidad de las neuronas piramidales gigantes en la corteza cerebral y el espesor de la capa piramidal interna de la corteza cerebral en los días 28, 56, 84 la densidad de las neuronas piramidales gigantes en corteza cerebral se redujo significativamente al comparar los controles ($107,6 \pm 6,2$, $131,6 \pm 4,6$ y $143,5 \pm 4,0$ respectivamente) con los casos ($84,96 \pm 2,1$, $109,8 \pm 7,3$ y $121,05 \pm 5,6$ respectivamente) ($p < 0,05$). El espesor de la capa piramidal interna de la corteza cerebral en los días 28, 84 y 56 fue significativamente mayor en el grupo diabético gestacional en comparación con el grupo control ($p < 0,05$). Este estudio muestra que la diabetes gestacional no controlada reduce la densidad de neuronas piramidales gigantes y el espesor interno de la capa piramidal en la corteza cerebral de las crías de rata.

PALABRAS CLAVE: Diabetes gestacional; Neuronas; Neuronas piramidales gigantes; Corteza cerebral; Crías de Rata.

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