

Morphometric study of cerebrum in fetuses of diabetic mothers

Khaksar, Z.^{1*}; Jelodar, G. A.¹ and Hematian, H.²

¹Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; ²Department of Veterinary, Islamic Azad University, Yasuj Branch, Yasuj, Iran

*Correspondence: Z. Khaksar, Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. E-mail: Khaksar@Shirazu.ac.ir

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Summary

In pregnant mothers maternal diabetes happens when the pancreas cannot produce enough insulin, so blood glucose increases in the mother and then in the fetus, resulting in several injuries in neonates. This study was conducted to evaluate the effects of maternal diabetes on fetal cerebrum. Sixteen adult female rats were divided in two equal groups. Diabetes was induced in one group by alloxan. Both groups became pregnant by natural mating. At days 17, 18, 19 and 20 of pregnancy, the cerebrum was collected from the fetuses of all rats, also the body weight and number of fetuses was measured. Various histological parameters were determined using routine histological techniques. Results revealed a significant decrease in the ratio of gray matter to white matter and also the number of cells in gray matter and white matter in all days. There was also a significant decrease in thickness of gray matter at day 20 of pregnancy in the cerebrum of fetuses of diabetic mothers (FDM) as compared with the control group. The body weight of FDM was significantly ($P<0.05$) more than that of the control group and the number of fetuses in FDM was significantly ($P<0.05$) less than the control group. Maternal hyperglycemia exhibited deleterious effects on cerebrum during fetal life, which affected: cell number, gray matter to white matter ratio and thickness of gray and white matter.

Key words: Maternal diabetes, Rat, fetus, Alloxan, Cerebrum

Introduction

The pancreas, by producing insulin, allows the body to use glucose efficiently. However, with diabetes, the pancreas insufficiently controls the insulin hormone, causing the blood sugar level to rise (Jones, 2001). In diabetic mothers during pregnancy, placental transport of glucose and other nutrients will be increased, resulting in fetal and neonatal Macrosomia (Persson and Hanson, 1998). Data indicate that pre-gestational maternal diabetes is strongly associated with multiple congenital abnormalities (Chung and Myriantopoulos, 1975). Maternal diabetes increased the incidence of major malformations (Cunningham *et al.*, 2005).

One of the mammalian systems that is clearly impaired in diabetes is the nervous system. Diabetes leads to a lack of sensation at the end of nerves (Cecil *et al.*, 2003). Atherosclerosis in the brain is one of the

prominent changes in diabetes. Studies have shown that obstruction of feeding vessels of nerves due to diabetes causes nerve bundles death and myelin destruction (Braunwald *et al.*, 2000). Also, decrease of nerve Na⁺, K⁽⁺⁾-ATPase activity is effective in the pathogenesis of human diabetic neuropathy (Scarpini *et al.*, 1993). An increased number of malformations occur in infants born from mothers with maternal diabetes involving the central nervous system (Aberg *et al.*, 2001) such as anencephaly, spina bifidia and hydrocephaly (Cunningham *et al.*, 2005).

Diabetes is associated with changes in both the barrier and transport functions of the cerebral microvessels. Structural changes in cerebral microvessels may account for some of the observed changes (Mooradian, 1997). The effect of diabetes on the brain suggests that it may lead to neurophysiological alterations, cognitive abnormalities, changes in both brain function and structure such as white matter

hyperintensities, and the gray matter density changes in type 1 diabetes, which suggests that persistent hyperglycemia and acute severe hypoglycemia have an impact on brain structure (Musen *et al.*, 2006). In addition, white matter microstructure deficits were seen in type 1 diabetes (Kodl *et al.*, 2008). Type 1 diabetes has had decreased gray matter and white matter in some parts of the cerebrum (Northam *et al.*, 2009). One study has shown that there is a relationship between neonatal diabetes mellitus and cerebellar hypoplasia or agenesis (Hoveyda *et al.*, 1999). Another study demonstrated that hyperglycemia prevents differentiation of cortical neurons, and causes oxidative stress in diabetic pregnancies of rat (Guleria *et al.*, 2006). Diabetes induce alteration in the dendritic morphology of cortical neurons (Martínez-Tellez *et al.*, 2005), hippocampal neuronal apoptosis (Li *et al.*, 2002), and disturbs the proliferation and cell death of neural progenitors (Gao and Gao, 2007).

The purpose of this investigation is to evaluate the possibility of congenital cerebral malformations in fetuses of diabetic rats at days 17, 18, 19 and 20 of pregnancy.

Materials and Methods

Sixteen adult female Sprague Dawley rats (200-250 g weight and 3–4-month-old) were acclimatized in an environmentally controlled room (temperature, $22 \pm 2^\circ\text{C}$, and 12 h light/12 h dark). Food and water were given *ad libitum*. In this study all experiments conducted on animals were in accordance with the guidance of the ethical committee for research on laboratory animals of Shiraz University. Animals were divided into two equal groups, experimental and control groups. Diabetes was induced in the experimental group by single intraperitoneal injection of alloxan tetrahydrate (Sigma, St. Louis, MO, USA) 145 mg/kg. The animals were fasted 12 h before and after alloxan injection. Rats with blood glucose 200-300 mg/dl, as well as with polydipsia, polyurea and polyphagia for at least one week were considered as diabetics and were chosen for the experiment (Szkudelski, 2001).

Female animals of both groups in oestrus stage were caged with male rat for mating. Mating was confirmed by vaginal plug observation (Turner and Bagnara, 1976). At days 17, 18, 19 and 20 of pregnancy, the fetuses were collected from both groups by surgery, and then the cerebrum was isolated from them. The Anastasia method was done by using diethyl ether. The body weight and number of fetuses were measured.

All tissue samples were immediately fixed in 5% buffered formalin fixative for histopathological investigations and subsequently embedded in paraffin. Sections (5 microns thickness) were stained with H&E and green masson's trichrome techniques. Sections were observed with an Olympus BX51 microscope for evaluation of histomorphometrical parameters such as:

- 1) Thickness of gray matter (μm)
- 2) Thickness of white matter (μm)
- 3) The number of cells in the gray matter per unit (mm^2)
- 4) The number of cells in white matter per unit (mm^2)
- 5) The ratio of gray matter to white matter

Thicknesses of gray matter and white matter were measured by ocular micrometer and Olympus BX51 light microscope, using Olysia software. The number of cells per unit (mm^2) in both white and gray matters and the ratio of gray matter to white matter were counted by ocular graticule and Olympus BX51 light microscope, using Olysia software. Analysis of particularly morphometric data was carried out with Student's t-test using SPSS program (Version 16).

Results

The fetal body weight changes of diabetic and control groups have been shown in Fig. 1. The mean of body weight in the fetuses of diabetic mothers (FDM) was significantly ($P < 0.05$) more than that of the control. Figure 2 demonstrates the number of fetuses in the FDM and control groups at all days. The number of fetuses of diabetic mothers (FDM) was significantly ($P < 0.05$) less than that of control group.

Table 1 demonstrates different para-

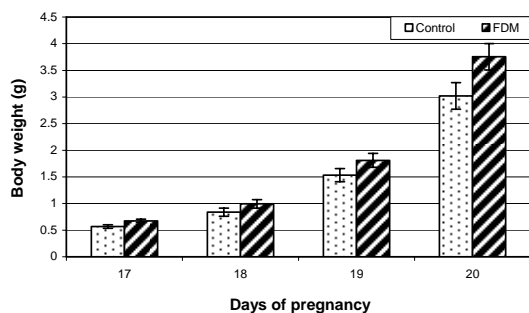


Fig. 1: Comparison of the body weight of fetuses of rats from normal (control) and diabetic mothers (FDM) at 17 to 20 days of pregnancy. The body weight of fetuses of diabetic mothers (FDM) increased significantly ($P<0.05$) compared to normal mothers (control) at days 17, 18, 19 and 20 of pregnancy

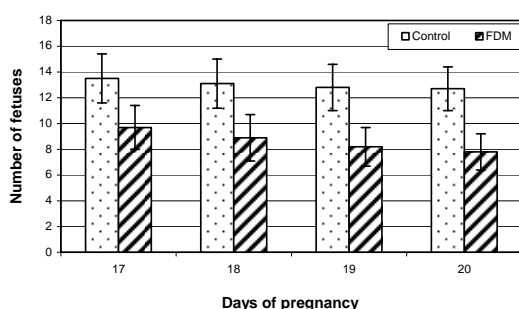


Fig. 2: Comparison of the number of fetuses of rats from normal (control) and diabetic mothers (FDM) at 17 to 20 days of pregnancy. The number of fetuses in diabetic mothers (FDM) decreased significantly ($P<0.05$) in comparison with normal mothers (control) at days 17, 18, 19 and 20 of pregnancy

meters of cerebrum of fetuses obtained from

diabetic (FDM) and control mothers at days 17, 18, 19 and 20 of pregnancy. The thickness of gray matter was decreased insignificantly in the FDM compared to that of the control, except at day 20 of pregnancy which was 432.23μ in FDM but 483.31μ in control group. The thickness of white matter was increased in FDM compared to that of the control at days 17, 18, 19 and 20 of pregnancy that was not significant. At day 17 of pregnancy, this value was 223.50μ in FDM but 222.66μ in the control group.

The number of cells in gray matter significantly ($P<0.05$) decreased in FDM compared to the control. At day 17 of pregnancy, this value was $19206.31/\text{mm}^2$ in FDM but $19823.80/\text{mm}^2$ in control group. The decrease as a percentage was 3.11, 3.8, 8 and 14% at days 17, 18, 19 and 20 of pregnancy, respectively. The number of cells in white matter decreased significantly in FDM compared to those of control ($P<0.05$). At day 17 of pregnancy, this value was $5122.73/\text{mm}^2$ in FDM but $5453.11/\text{mm}^2$ in the control group. The decrease in the number of cells in white matter as a percentage at days 17, 18, 19 and 20 was 6.07, 3.41, 3.93 and 9.95%, respectively. The ratio of gray matter to white matter was decreased significantly ($P<0.05$) in FDM compared to that of the control at days 17, 18, 19 and 20 of pregnancy. The percentage of reduction was 2.36, 3.14, 3.49 and 4.85% at days 17, 18, 19 and 20 of pregnancy, respectively.

Table 1: Comparison of FDM and control parameters of the cerebrum at 17, 18, 19 and 20 days of pregnancy

Group	Age (day)							
	17		18		19		20	
	FDM	Control	FDM	Control	FDM	Control	FDM	Control
TGM (μ)	426.94 ± 25.22	431.42 ± 22.50	436.63 ± 27.61	443.23 ± 24.24	442.67 ± 47.35	465.72 ± 45.90	432.23 ± 42.17	483.31 $\pm 40.05^*$
TWM (μ)	223.50 ± 11.24	222.66 ± 10.23	241.63 ± 11.58	240.74 ± 10.49	277.09 ± 26.03	266.51 ± 21.36	313.35 ± 20.43	302.54 ± 25.56
NGM (n/mm^2)	19206.31 ± 1072.96	19823.80 $\pm 1029.14^*$	22722.44 ± 1610.28	23620.07 $\pm 1514.81^*$	24469.24 ± 1122.47	26597.03 $\pm 1287.85^*$	21217.63 ± 1644.80	24672.19 $\pm 1730.25^*$
NWM (n/mm^2)	5122.73 ± 465.23	5453.11 $\pm 471.02^*$	8331.75 ± 539.06	8625.32 $\pm 532.12^*$	11577.55 ± 892.28	12050.22 $\pm 914.79^*$	9668.04 ± 602.04	10737.43 $\pm 617.37^*$
GWR	2.07 ± 0.12	2.12 $\pm 0.11^*$	1.85 ± 0.11	1.91 $\pm 0.10^*$	1.66 ± 0.12	1.72 $\pm 0.11^*$	1.57 ± 0.12	1.65 $\pm 0.14^*$

FDM: Fetuses of diabetic mothers, TGM: Thickness of gray matter, TWM: Thickness of white matter, NGM: Number of cells in gray matter, NWM: Number of cells in white matter, and GWR: Ratio of gray matter to white matter. Values are demonstrated with mean \pm SD. Significant difference between FDM and control demonstrated with * sign ($P<0.05$)

Discussion

The body weight of fetuses of diabetic mothers was significantly more than that of the control (Macrosomia), which is due to increase in placental transport of glucose and other nutrients (Jones, 2001). In a previous study, the body weight of offspring of diabetic rats was increased significantly (Khaksar *et al.*, 2010).

The number of fetuses of diabetic mothers was significantly less than that of control group. Moley *et al.* (1998) reported that hyperglycemia induces apoptosis in pre-implantation embryos through cell death effectors pathways; their results indicated that hyperglycemic conditions, either *in vivo* or *in vitro*, modulate the expression of an apoptosis regulatory gene as early as the pre-implantation blastocyst stage in the mouse. Stillbirths are a phenomenon found in pregnancies complicated by pregestational diabetes (Cunningham *et al.*, 2005).

The thickness of white matter in cerebrum was increased insignificantly in FDM compared to control group at days 17 to 20 of pregnancy, whereas the thickness of gray matter was decreased in FDM compared to control at days 17 to 20 of pregnancy which was significant only at day 20. Neuropathy of numerous nerves like sciatic nerve has been reported in diabetics (Artico *et al.*, 2002). Malformations in this region of the brain may occur due to neuropathy. Diabetes mellitus is associated with moderate cognitive deficits and neurophysiological and structural changes in the brain, a condition that may be referred to as diabetic encephalopathy. The emerging view is that the diabetic brain features many symptoms that are best described as "accelerated brain ageing" (Biessels *et al.*, 2002). Maternal diabetes leads to white matter hyperintensities and gray matter density changes in fetus (Musen *et al.*, 2006). In addition, white matter microstructure deficits were seen in type 1 diabetic subjects which correlate with impaired performance on neurocognitive tests that are thought to be associated with white matter function (Kodl *et al.*, 2008). Northam *et al.* (2009) have shown type 1 diabetic subjects, relative to control subjects, had decreased gray matter in bilateral

thalami and right parahippocampal gyrus and insular cortex. White matter was decreased in bilateral parahippocampi, the left temporal lobe, and middle frontal area (Northam *et al.*, 2009). A study has shown neonatal diabetes mellitus may cause cerebellar agenesis or hypoplasia (Hoveyda *et al.*, 1999).

Maternal diabetes leads to fetal hyperbilirubinemia (Cunningham *et al.*, 2005), that could result in an encephalopathy named Kernicterus (Murray *et al.*, 2003). Also, the reduction in Na⁺, K⁽⁺⁾-ATPase activity in diabetic nerves may be an important factor in the pathogenesis and self-maintenance of human diabetic neuropathy (Scarpini *et al.*, 1993). Guleria *et al.* (2006) have shown that hyperglycemia inhibits retinoic acid which prevents differentiation of cortical neurons, and causes oxidative stress in a rat model of diabetic pregnancy. Retinoic acid applied at a physiological concentration significantly decreased hyperglycemia-induced oxidative stress and thus supported the antioxidant defense system (Guleria *et al.*, 2006). Therefore, these reasons could be considered for malformation of this region due to maternal diabetes.

Table 1 demonstrates a decrease in the number of cells in gray matter and white matter in FDM compared to the control group. Hyperglycemic condition disturbs the proliferation and cell death of neural progenitors in mouse embryonic spinal cord (Gao and Gao, 2007). Insufficient expression of genes that regulate the viability of the progenitor cells is responsible for the apoptosis (Chappell *et al.*, 2009).

Hyperglycemia effectively makes more substrate available for aerobic glycolysis in the brain, leading to acidosis (Biessels *et al.*, 1994) and enhanced oxygen free radical formation by reduction in levels of protective endogenous antioxidants (Baydas *et al.*, 2002). These radicals contribute to increased neuronal death by oxidizing proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes (Hawkins and Davies, 2001). 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). Maternal diabetes induces some changes in hippocampus neuronal structure and density. Statistical analysis show a significant decrease in neuronal density (ND) in neonates from diabetic mothers compared to the control (Tehranipour and Khakzad, 2008); those changes can be due to cell apoptosis, as Li *et al.* (2002) have shown that a duration-related apoptosis-induced neuronal loss in the hippocampus occurs in type 1 diabetes and is associated with cognitive impairment. Martínez-Tellez *et al.* (2005) explained that diabetes mellitus may in part affect the dendritic morphology in the limbic structures, such as the prefrontal cortex, occipital cortex, and hippocampus, which are implicated in cognitive disorders.

In conclusion, maternal diabetes has significant deleterious effects on cerebrum and leads to a decrease in the number of cells and the ratio of gray matter to white matter, and changes thicknesses of white and gray matter in the cerebrum as well.

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