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Morphological changes in the brachial enlargement of the spinal cord in offspring of diabetic rat

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Summary

This study was conducted to evaluate the effects of maternal diabetes on fetal spinal structure, especially in brachial enlargement. Sixteen adult female rats were divided into two groups. Diabetes was induced in one group by alloxan agent. Both groups became pregnant by natural matting. On days 7, 14, 21 and 28 after birth, the brachial enlargement of the spinal cord was collected from offspring of all rats and the weight of neonates was measured. Various histological parameters were determined using histological techniques. The results revealed a significant decrease in transverse spinal diameter and number of neurons of gray matter and an increase in vertical spinal diameter in spinal cord of offspring of diabetic mothers (ODM) as compared with the control group. The body weight of ODM was significantly more than that of the control group ($P<0.05$). Maternal hyperglycemia exhibited deleterious effects on spinal cord, especially brachial enlargement during fetal life which remained persistent during postneonatal period.

Key words: Maternal diabetes, Rat, Offspring, Alloxan, Brachial enlargement

Introduction

Pancreas by producing insulin, allows the body to use glucose efficiently. However, with diabetes, the pancreas insufficiently controls the hormone insulin, causing blood sugar levels to rise (Jones, 2001). In diabetic mothers during pregnancy placental transport of glucose and other nutrients will be increased, due to an increased availability at the maternal site, resulting in their increase in fetal and neonatal Macrosmia (Persson and Hanson, 1998). The elevated serum glucose concentration in mother, accompanying hyperglycemia in the fetus, lead to degranulation of the fetal β-cells, resulting in fetal hypoinsulinemia. Indeed, the majority of the newborn of a badly controlled diabetic mother (blood glucose>16.7 mmol/1) shows degranulation of pancreatic β-cells (Van Assche *et al*., 1983). The risk for diabetes is significantly higher in the offspring of mothers who have non-insulin-dependent diabetes (Knowler *et al*., 1985). In addition, maternal diabetes increases the risk of hypoglycemia and other chemical imbalance as low calcium and magnesium levels (Jones, 2001). Data indicate that pre-gestational maternal diabetes is associated with strong teratogenic effects on the kidney, urinary tract and heart and also strongly associated with multiple congenital abnormalities (Chung and Myrianthopoulos, 1975).

One of the mammalian systems that clearly impaired in diabetes is nervous system. Diabetes leads to senseless at the end of nerves (Cecil *et al*., 2003). Atherosclerosis in 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 (Harrison *et al*., 2000). Diabetes decreases tonic and phasic pains sensitivity in animals (Jelodar and Akbari, 1996). An increased number of malformations occur in infants born from mothers with maternal diabetes involving the central nervous system (CNS), the spinal

column, the ribs and the urinary tracts (Martinez-Frais *et al*., 1998; Aberg *et al*., 2002; Farrell *et al*., 2002). Specific types of anomalies in CNS which linked to maternal diabetes are anencephaly, spina bifidia and hydrocephaly (Cuningham *et al*., 2005). Maternal diabetes-induced hyperglycemia and acute intracerebral hyperinsulinism reduces fetal brain neuropeptide Y concentrations (Singh *et al*., 1997). Commonly, fetuses with CNS anomalies, or exposed to adverse conditions which may affect CNS functioning, take longer to habituate, or fail to habituate (Hepper and Leader, 1996). The organization of behavioural states is poorer in fetuses of diabetic mothers than non-diabetic mothers (Mulder and Visser, 1991). 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).

The purpose of this investigation is to evaluate the possibility of congenital spinal malformation in brachial enlargement of offspring of diabetic rats in four first week of their life.

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 ± 2°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 ethical committee for research on laboratory animals of Shiraz University.

The animals were divided into two equal groups, experimental and control groups. Diabetes was induced in 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 (Szkudelski, 2001). Rats with blood glucose 200-300 mg/dl as well as with polydipsia, polyurea and polyphagia, which last for at least one week, were considered as diabetics and selected for the experiment. 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). All the newborn offspring of both groups were kept at the same condition in animal house.

On days 7, 14, 21 and 28, six offspring rats, in diabetic and control groups were anaesthetized (using diethyl ether) and sacrified. For histopathological examination, all tissue samples were fixed in 5% buffered formalin and then embedded in paraffin. Sections (5 microns thickness) were stained with haematoxylin and eosin and Green Masson's trichrome techniques and then observed with Olympus BX51 microscope for evaluation of histomorphometrical parameters such as:

1) Transverse and vertical diameters of spinal cord (μm), 2) Transverse and vertical diameters of central canal of spinal cord (μm), 3) The number of neurons per unit (mm^2) in the gray matter, 4) The number of neuroglial cells in gray and white matters separately per unit (mm^2), and 5) The ratio of gray matter to white matter.

Transverse and vertical diameters of spinal cord and central canal were measured by ocular micrometer and Olympus BX51 light microscope using Olysia software. The number of neurons and neuroglial 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.

Results

The neonatal body weight changes in diabetic and control groups have been shown in Fig. 1. The mean body weight of offspring of diabetic mothers was

significantly (P<0.05) more than that of the control group.

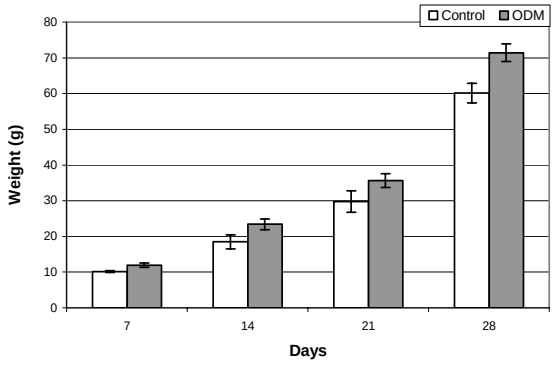

Fig. 1: Comparison of the body weight of offspring rats from normal and diabetic mothers (ODM) on days 7, 14, 21 and 28 postneonatal

Table 1 demonstrates different parameters of brachial enlargement of the spinal cord from offspring of diabetic mothers (ODM) and control mothers on days 7, 14, 21 and 28 after birth. The transverse spinal diameter was decreased in ODM as compared with the control group and these differences were significant (P<0.05) except for day 7 after birth which was 1928.75 µ in ODM but 1935.75 µ in the control group. The percentage of reduction was 0.4, 5, 9.3 and 9.1% for days 7, 14, 21 and 28 postneonatal, respectively. The vertical spinal diameter was significantly (P<0.05) increased in ODM as compared with the control group. The increase on days 7, 14, 21 and 28 postneonatal as percentage was 3.9, 9, 8.7 and 14.5%, respectively. The transverse diameter of the central canal was decreased in ODM as compared with the control group but this reduction was not significant. On day 7 after birth, this value was 64.85 µ in ODM but 67.50 µ in control group. The decrease as percentage was 3.9, 3.2, 2.5 and 4.5% for days 7, 14, 21 and 28 postneonatal, respectively. The vertical diameter of the central canal was increased in ODM as compared with the control group and this increase was insignificant. The increase as percentage on days 7, 14, 21, 28 was 3.5, 4.2, 3.4 and 4%, respectively. The number of neurons was decreased in ODM as compared with the control group and this decrease was not significant except on day 7 after birth. On day 7, this value was 291/mm^2 in ODM but 321/mm^2 in the

Table 1: Comparison of different parameters of brachial enlargement of the spinal cord on days 7, 14, 21 and 28 after birth between ODM and control groups

	Age (day)							
	7		14		21		28	
Group	ODM	Control	ODM	Control	ODM	Control	ODM	Control
TDS (µ)	1928.75 ±66.90	1935.75 ±75.86	2300.01 ±17.33	2420.50 ±82.75*	2611.75 ±161.40	2880.50 ±148.40*	3013.75 ±100.39	3318.25 ±162.08*
VDS (µ)	1616.51 ±54.43	1555.5 ±45.79*	1821.50 ±167.00	1673.00 ±42.94*	1960.00 ±80.65	1803.50 ±56.29*	2331.61 ±72.95	2036.63 ±67.17*
TDC (µ)	64.85 ±6.65	67.50 ±3.87	69.72 ±18.40	72.00 ±7.05	57.75 ±11.69	59.25 ±10.80	54.50 ±4.05	57.00 ±2.56
VDC (µ)	93.75 ±10.00	90.25 ±2.99	100.25 ±5.03	96.20 ±15.82	116.60 ±7.91	112.75 ±12.82	122.75 ±10.85	118.00 ±22.07
GWR	1.66 ±0.008	1.66 ±0.009	1.58 ±0.024	1.60 ±0.013	1.45 ±0.031	1.45 ±0.02	1.39 ±0.023	1.39 ±0.16
NNG (n/mm^2)	291.00 ±3.59	321.00 ±5.17*	230.25 ±10.32	239.40 ±6.74	253.75 ±7.60	257.20 ±3.02	309.72 ±6.47	312.37 ±10.07
NNGL (n/mm^2)	949.75 ±5.56	944.35 ±1.39	880.35 ±3.57	868.75 ±17.68	808.65 ±17.06	802.60 ±29.59	650.25 ±9.49	649.52 ±23.66
NNGW (n/mm^2)	832.55 ±6.81	833.60 ±6.78	884.30 ±12.05	889.30 ±18.95	941.40 ±8.30	945.40 ±18.10	1006.25 ±7.86	1008.00 ±7.44

ODM: offspring of diabetic mothers, TDS: transverse diameter of the spinal cord, VDS: vertical diameter of the spinal cord, TDC: transverse diameter of the central canal, VDC: vertical diameter of the central canal, GWR: ratio of gray matter in relation to white matter, NNG: number of neurons of gray matter, NNGL: numbers of neuroglial cell of gray matter, and NNGW: numbers of neuroglial cell of white matter. Values are demonstrated as mean ± SD. * Shows significant difference between ODM and control groups, (P<0.05)

control group. The percentage of reduction was 9.3, 3.8, 1.3 and 0.8% for days 7, 14, 21 and 28 postneonatal, respectively. The number of neuroglial cells in gray matter insignificantly increased in ODM as compared with the control group. The increase as percentage on days 7, 14, 21, 28 was 0.57, 1.3, 0.7 and 1.5%, respectively. The number of neuroglial cells in white matter insignificantly decreased in ODM as compared with the control group. On day 7 after birth, this value was 832.55/mm^2 in ODM but 833.60/mm^2 in the control group. The percentage of reduction was 0.12, 0.56, 0.4 and 0.2% for days 7, 14, 21 and 28 postneonatal, respectively. The ratio of gray matter to white matter was decreased in ODM as compared with the control group on day 14 after birth which was not significant (1.2%), however no differences were found between ODM and control groups on days 7, 21 and 28 postneonatal. Figures 2, 3 and 4 demonstrate the comparison of size and the cell number of brachial enlargement of the spinal cord in both control (A) and ODM (B) groups on day 14 after birth.

Discussion

The body weight of infants of diabetic mothers was significantly more than that of control group (macrosomia), which is due to increase in placental transport of glucose and other nutrients (Jones, 2001). In macrosomia, neonates have additional fats in shoulders and trunk (Cuningham *et al*., 2005).

The transverse spinal diameter in brachial enlargement of the spinal cord was decreased in ODM as compared with the control group on days 7, 14, 21 and 28 after birth, whereas the vertical spinal diameter in

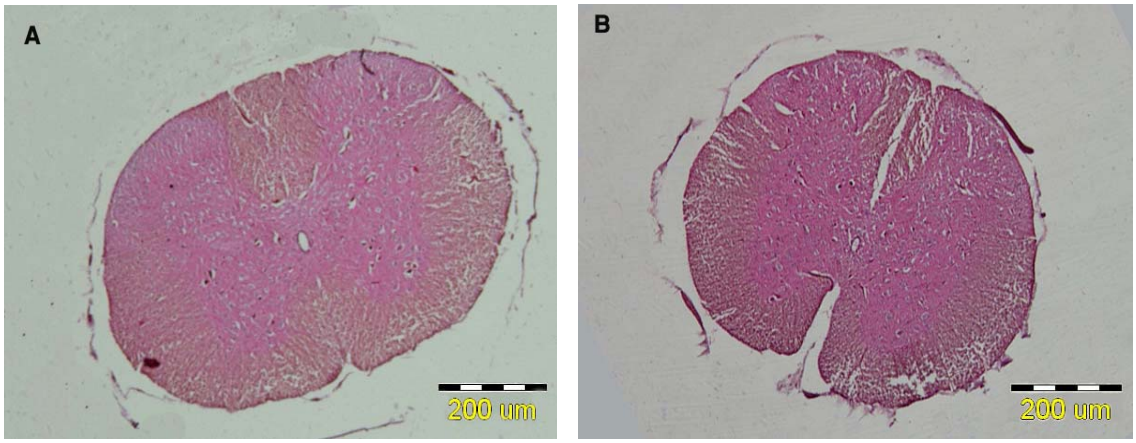

Fig. 2: Comparison of the size of transverse section in brachial enlargement of the spinal cord 14 days after birth in control (A) and ODM (B) groups, (H&E)

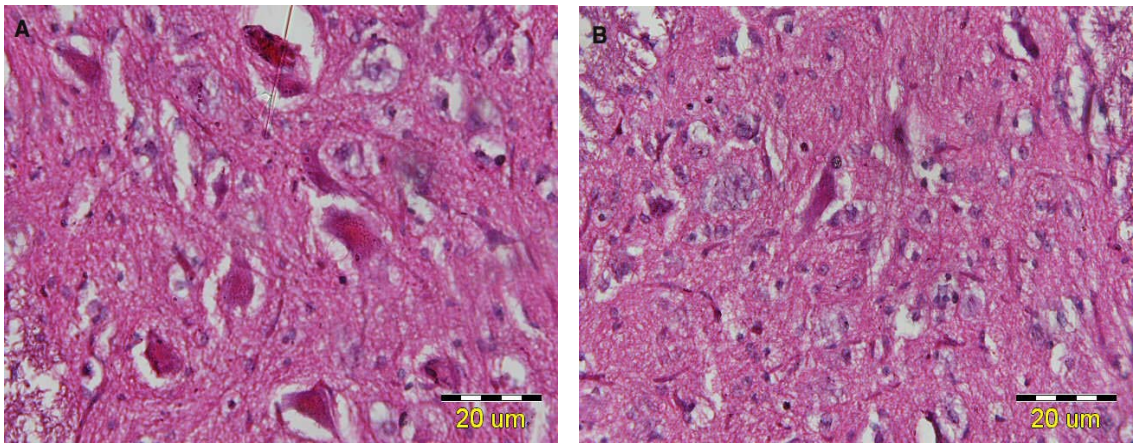

Fig. 3: Comparison of the neurons number of gray matter in brachial enlargement of the spinal cord 14 days after birth in control (A) and ODM (B) groups, (H&E)

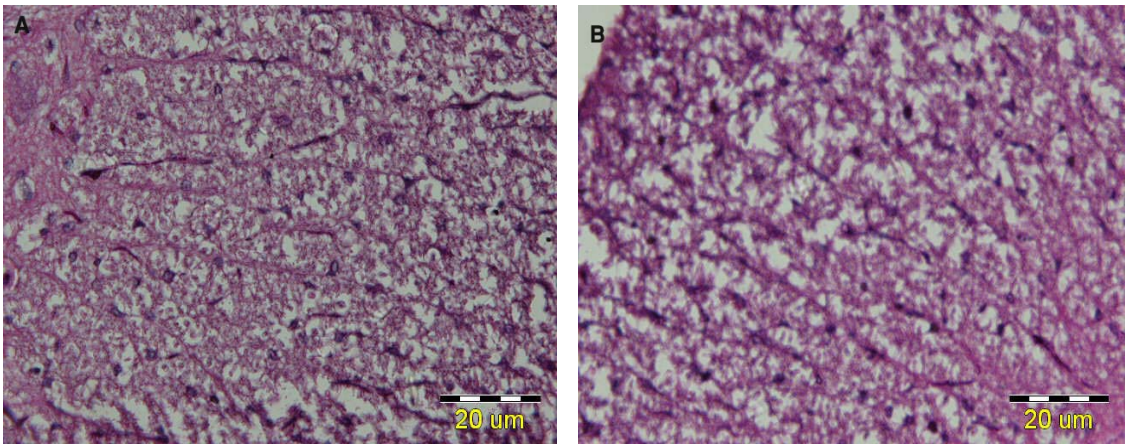

Fig. 4: Comparison of the cell number of white matter in brachial enlargement of the spinal cord 14 days after birth in control (A) and ODM (B) groups, (H&E)

brachial enlargement of the spinal cord was increased in ODM in comparison with the control group. Therefore, maternal diabetes results in malformation of this region of the spinal cord.

Neuropathy of numerous nerves like sciatic nerve has been reported in ODM (Artico *et al*., 2002; Guyton and Hall, 2006). Malformations in this region of the spinal cord may occur due to neuropathy. Table 1 demonstrates a decrease in the number of neurons in gray matter in ODM in comparison with the control group. On day 7 after birth, there is a significant ($P<0.05$) difference between ODM and control groups. In diabetic pregnancy, maternal glucose transport to fetal blood via the placenta (Jones, 2001) and increase in fetal blood glucose may result in diabetic neuropathy in fetus, as diabetes leads to neuropathy in adult (Guyton and Hall, 2006). Maternal diabetes leads to white matter hyperintensities and gray matter density changes in fetus (Musen *et al*., 2006). Hyperglycemic condition disturbs the proliferation and cell death of neural progenitors in mouse embryonic spinal cord (Gao and Gao, 2007). For other parameters, there were no significant differences between ODM and control groups. Therefore, probably diabetes could not affect the transverse and vertical diameter of the central canal, ratio of gray matter to white matter and the number of neuroglial cells in the gray and white matters in brachial enlargement.

In conclusion, maternal diabetes has significant deleterious effects on brachial enlargement of spinal cord and leads to decrease in the number of neurons and change in the shape of the spinal cord.

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