



GENDER-DEPENDENT MICROMORPHOMETRIC ALTERATIONS IN THE BRAIN OF DIABETIC RATS

H. HEMATIAN

Department of Veterinary Sciences, Yasooj Branch, Islamic Azad University,
Yasooj, Iran

Summary

Hematian, H., 2014. Gender-dependent micromorphometric alterations in the brain of diabetic rats. *Bulg. J. Vet. Med.*, **17**, No 2, 88–95.

This study was conducted to evaluate the effects of diabetes on the brain structure in rats and to compare the brain alterations in the two genders. Twenty adult female and the same number of male Sprague Dawley rats were divided in four groups (5 males and 5 females in each). Diabetes was induced in 3 groups by alloxan (single intraperitoneal injection of 145 mg/kg alloxan tetrahydrate). Rats with blood glucose above 200 mg/dL (11.1 mmol/L) for at least one week were considered as diabetic and were chosen for experiment. All animals were housed under the same conditions but for different periods of time (30, 60 and 90 days). Body weights were measured before and after the experiment. After the experiment, animals were anaesthetised, sacrificed and brains were collected to determine various histological parameters using histological techniques. Results revealed a decrease in male and female diabetics' final body weight compared to that of controls. The thickness and the number of cells in the gray matter and the white matter were reduced in all test groups. In females, the thickness of white matter and the cell numbers of the gray and white matters were decreased more than in male rats. According to our results, diabetes exhibited deleterious effects on male and female rat brains, although these effects were more serious in female rats.

Key words: alloxan, brain, diabetes, gender, rat

INTRODUCTION

Pancreas produces insulin and 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). One of the mammalian systems that is clearly impaired in diabetes is the nervous system (Cecil *et al.*, 2003). Neuropathy and encephalopathy are important complications of diabetes (Pesaresi *et al.*, 2010).

Studies have shown the alterations in both function and structure of the brain (Musen *et al.*, 2006); as well as decrease in cell number of the brain (Khaksar *et al.*, 2012).

It has been reported that gender and diabetes had significant effects on mortality from hypoxic-ischaemic and extensive brain damage (Vannucci *et al.*, 2001). Although low levels of testosterone in

men have been shown to be associated with diabetes (Kapoor *et al.*, 2006) in females it protects diabetics against the neuropathy (Pesaresi *et al.*, 2011). Also, it has been demonstrated that females are more heavily affected by some diseases due to interaction of the sex hormones (Brabin, 2002). Females are more susceptible to neurodegenerative disorders related to higher myelin protein turnover than males (Massella *et al.*, 2012). So, observations have demonstrated that the gender might affect the diabetes brain effects due to differences of the sex hormones.

This investigation was performed to evaluate the gender effects on the micro-morphometric alterations of the brain in diabetic rats.

MATERIALS AND METHODS

Animals

Twenty adult male (250–270 g body weight; 3–4 months of age) and twenty adult female Sprague Dawley rats (210–230 g body weight; 3–4 months of age) were acclimatised 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 animal experiments were in accordance with the guidance of the Ethical Committee for Research on Laboratory Animals of Shiraz University. Animals were divided into four equal groups. Three groups were experimental (T1, T2 and T3) and one group was control (C). Each group contained 10 rats (5 male and 5 female).

Experimental design

Diabetes was induced in the experimental groups by single intraperitoneal injection of alloxan tetrahydrate (Sigma, St. Louis,

MO, USA) at a dose of 145 mg/kg. Alloxan destroys the β -cells of the pancreas and induces diabetes type I. The animals were fasted 12 h before and after alloxan injection (Szkudelski, 2001). Rats with blood glucose above 11.1 mmol/L as well as with polydipsia, polyuria and polyphagia for at least one week (such as increased moistness of the cage bottom, increased water and food consumption compared to time before the injection), were considered as diabetic and were chosen for experiment. The blood glucose levels were measured by collecting blood from the tail and measuring by a glucometer.

All the animals were kept at the same condition in animal house. Animals of group T1 were kept for 30 days, group T2 – for 60 days and group T3 – for 90 days. The behaviour of all rats was also evaluated during the investigation, as an important feature describing the presence of brain damage in the diabetic groups. After the experiment, animals were anaesthetised (by using diethyl ether) and sacrificed. Then the brain was collected from all rats. Animals of control group (C) were sampled as others. The weights of all rats were measured before and after the experiment.

For histopathological investigations, all tissue samples were fixed in 5% buffered formalin fixative for 48–72 h and subsequently dehydrated in alcohol series, cleared in 100% xylene and embedded in paraffin. Sections were taken by a microtome (5 μ m thickness) and stained with haematoxylin-eosin (Luna, 1968).

Morphometry of the brain

Sections were observed with Olympus BX51 microscope for evaluation of the following histomorphometrical parameters:

- Thickness of gray matter (μm).
- Thickness of white matter (μm)
- Number of cells in the gray matter per unit (mm^2).
- Number of cells in white matter per unit (mm^2).
- Ratio of gray matter to white matter.

Thicknesses of gray 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 matter, and the gray to white matter ratio were counted by ocular graticule and Olympus BX51 light microscope using Olysia software.

Statistical analysis

Analysis of particularly morphometric data was performed with Student's *t*-test using SPSS software.

RESULTS

Blood glucose of males was increased significantly ($P < 0.05$) in diabetics compared to controls (15.1 ± 0.7 mmol/L vs. 4.8 ± 0.2 mmol/L). Additionally, in females, blood glucose of diabetic groups was increased significantly ($P < 0.05$) compared to control groups (14.7 ± 0.6 mmol/L vs. 4.7 ± 0.2 mmol/L).

The body weights of diabetic and control groups of rats of both genders before and after the experiment are shown in Figs. 1 and 2, respectively. The body weights of diabetic rats from both genders were reduced after the experiment compared to baseline weights and the mean body weight of group T3 was significantly ($P < 0.05$) lower than that of both male and female control rats.

The behaviour of all animals was evaluated meticulously, it has been determined that test animals were confused and exhibited dizziness and almost malaise; as

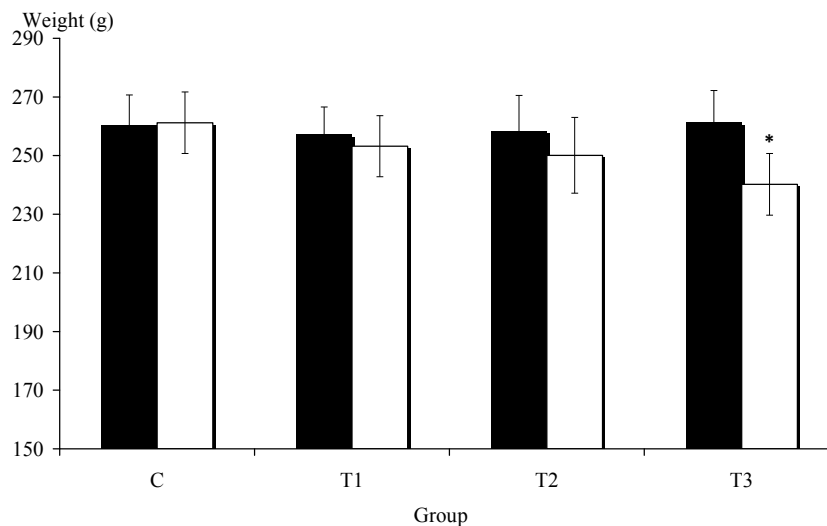


Fig. 1. Body weight of test (T1, T2, T3) and control group (C) of male rats before (black bars) and after (white bars) the experiment. Significant difference between test and control groups is indicated with an asterisk ($P < 0.05$).

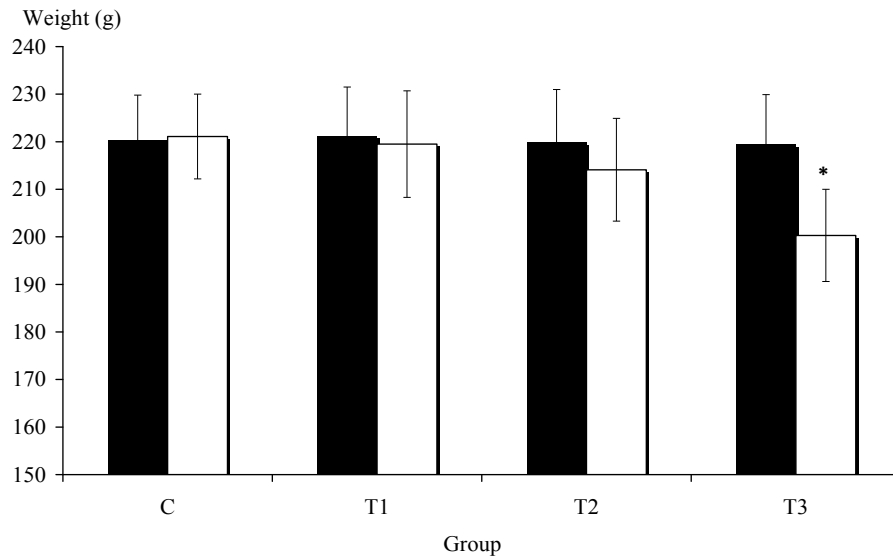


Fig. 2. Body weight of test (T1, T2, T3) and control group (C) of female rats before (black bars) and after (white bars) the experiment. Significant difference between test and control groups is indicated with an asterisk ($P<0.05$).

Table 1. Thickness of gray matter (TGM), thickness of white matter (TWM), number of cells in gray matter (NGM), number of cells in white matter (NWM), and gray to white matter ratio (GWR) in test (T1, T2 and T3) and control (C) groups of male rats. Values are presented as mean±SD (n=5). Significant differences between test and control groups are indicated with an asterisk ($P<0.05$).

Groups	C	T1	T2	T3
TGM (µm)	514.37±43.14	509.22±41.51	498.65±39.91	487.25±37.19
TWM (µm)	592.32±36.39	587.82±32.71	568.88±33.21	545.12±39.27
NGM (per mm ²)	2632.1±149.6	2604.6±136.2	2538.3±138.3	2422.3±115.9
NWM (per mm ²)	1772.5±56.3	1749.8±54.9	1703.1±63.7	1655.9±58.2*
GWR	0.84±0.09	0.84±0.08	0.84±0.11	0.85±0.12

the duration of diabetes increased these disorders became more serious.

Table 1 demonstrates different brain parameters in diabetic and control male rats. The thicknesses of gray and white matter, and the cell numbers of gray matter were insignificantly decreased in the test groups (T1, T2 and T3) compared

to control (Group C). The number of cells in the white matter was significantly ($P<0.05$) decreased in group T3 compared to that of control (1655.9 vs 1772.5), whereas this parameter showed an insignificant decrease in other groups compared to the control group (C).

Table 2. Thickness of gray matter (TGM), thickness of white matter (TWM), number of cells in gray matter (NGM), number of cells in white matter (NWM), and gray to white matter ratio (GWR) in test (T1, T2 and T3) and control (C) groups of female rats. Values are presented as mean±SD (n=5). Significant differences between test and control groups are indicated with an asterisk (P<0.05).

Groups	C	T1	T2	T3
TGM (µm)	511.25±38.94	499.48±43.23	482.04±34.74	469.54±32.28
TWM (µm)	585.67±32.27	567.43±38.37	553.08±29.30	518.12±31.44*
NGM (per mm ²)	2627.2±138.7	2568.7±129.5	2485.8±118.4	2382.4±110.7*
NWM (per mm ²)	1791.2±52.2	1751.4±45.5	1663.5±49.3*	1607.4±44.1*
GWR	0.83±0.09	0.83±0.08	0.84±0.12	0.85±0.14

Table 2 presents the same parameters of brain from diabetic and control female rats. The thickness of gray matter was decreased insignificantly in the test groups. The thickness of white matter in group T3 showed a significant (P<0.05) reduction compared to control (518.12 vs 585.67). Also, the number of cells in the gray matter was significantly (P<0.05) decreased in the group T3 vs control group (2382.4 vs 2627.2). The number of cells in the white matter have shown a significant (P<0.05) decrease in the T2 (1663.5) and T3 groups (1607.4) compared to the control group (1791.2).

DISCUSSION

It has been demonstrated that diabetes is accompanied by polyuria and consequently dehydration and weight loss (Marliss *et al.*, 1982; Kuzuya *et al.*, 2002). Our data are in agreement with these findings, as body weights of diabetic animals were significantly lower than those of male and female controls.

The thickness of gray matter and white matter were decreased in diabetics of the two genders compared to controls. The cell numbers of gray and white matter was also decreased. It could be therefore

affirmed that diabetes altered the thickness and cell number in the gray and white matter of this region. Khaksar *et al.* (2012) have determined that maternal diabetes had serious effects on the brain leading to decrease in the number of neurons and altered brain structure. One study has shown that hyperglycaemia is effective in producing more substrate for aerobic glycolysis in the brain, leading to acidosis (Biessels *et al.*, 1994) and enhanced oxygen free radical formation consequently to reduction in levels of protective endogenous antioxidants (Baydas *et al.*, 2002). These radicals contribute to increased neuronal death by oxidising proteins, DNA damage and lipoperoxidation of cellular membranes (Hawkins & Davies, 2001). Northam *et al.* (2009) have shown several neuropathological processes including gliosis, demyelination, and altered osmolarity in the brain of diabetics (Northam *et al.*, 2009).

The behaviour evaluations of the experimental animals have shown mental and cognitive disorders. Kodl *et al.* (2008) have demonstrated white matter microstructure deficits in type 1 diabetic subjects, which correlated with impaired performance on neurocognitive tests that are thought to be associated with white matter function. Li *et al.* (2002) have shown that

a duration-related apoptosis-induced neuronal loss in the hippocampus occurred in diabetes type 1, associated with cognitive impairment. It has been also determined that diabetes mellitus was linked to moderate cognitive deficits and neurophysiological and structural changes in the brain, a condition that may be referred to as diabetic encephalopathy described as "accelerated brain ageing" (Biessels *et al.*, 2002).

The thickness of white matter and the number of cells in gray and white matter have shown more alterations in female rats compared to the males, as the decrease in thickness of white matter and the cell number of gray matter in group T3 and the white matter cell numbers in group T2 were significant ($P < 0.05$) only in the females. Also, the number of cells in the white matter was significantly decreased ($P < 0.05$) in group T2 compared to the control group only in females. So, diabetes induced more significant effects in the brain of females than of males. Kapoor *et al.* (2006) reported that low levels of testosterone in men were associated with type 2 diabetes, visceral adiposity, dyslipidaemia and metabolic syndrome. This investigation also demonstrated testosterone replacement therapy in men reduced insulin resistance and improved glycaemic control in hypogonadal men with type 2 diabetes. One investigation has demonstrated that gonadectomy in female, but not in male diabetic animals, protected against the neuropathy (Pesaresi *et al.*, 2011).

The observations in this experiment suggested that in male rats, neuroactive steroids are protective agents and that their levels in peripheral and central nervous system are strongly affected by the disease (Pesaresi *et al.*, 2010). In the central nervous system, dehydroepiandro-

sterone, an intermediate in the biosynthesis of androgens (Gravanis *et al.*, 2012), protects the hippocampus from diabetic damages (Aragno *et al.*, 2002). Neuroactive steroids, such as testosterone and its metabolites modulate the expression of key transcription factors for Schwann cell function, regulate Schwann cell proliferation and promote the expression of myelin proteins involved in the maintenance of myelin multilamellar structure (Roglio *et al.*, 2008), so testosterone could perform the same role in the brain of males and protect this organ from the harmful effects of diabetes. Additionally, it must be cited that females are more susceptible to many diseases (Klein *et al.*, 2010; Robinson *et al.*, 2011) including diabetes possibly because of the effect of female sex hormones on disease susceptibility and progression (Brabin, 2002).

Females suffer more from neurodegenerative disorders than males. It has been reported that oligodendrocytes and the content of several myelin proteins in white tracts were higher in males than in females, whereas the lifespan of oligodendrocytes was shorter in females than in males, thus suggesting a greater myelin turnover in females than in males. Thus females may be more vulnerable to higher myelin protein turnover (Massella *et al.*, 2012).

Taken together, the observations supported the hypothesis that diabetes affects the brain more seriously in the females than males; however, it seems necessary to carry out further studies on the hormones and metabolites related to this subject.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Yasooj Branch of Islamic Azad University.

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Paper received 20.05.2013; accepted for publication 11.09.2013

Correspondence:

H. Hematian
Department of Veterinary Sciences,
Yasooj Branch,
Islamic Azad University,
Yasooj, Iran
e-mail: Dr.hoomanhematian@yahoo.com