

Gestational Diabetes Reduces Pancreatic Beta Cells in Rat Offspring

La Diabetes Gestacional Reduce las Células Beta Pancreáticas en Crías de Ratas

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SUMMARY: Previous study has shown the adverse effects of gestational diabetes on hippocampal and spinal cord neuronal density in animal model. This study was conducted to determine the effect of gestational diabetes on beta cells in rat pancreas in early postnatal life. In this experimental study, 10 dams randomly allocated into control and diabetic groups on day 1 of gestation. Five dams in diabetic group received 40 mg/kg/BW of streptozotocin (intraperitoneally) and control animals received normal saline. Six of 28 and 56-day-old offspring of each gestational diabetes mellitus and controls were randomly scarified and sections were taken from the pancreas and stained using Gomorra's method. The density of beta cells and number and area of pancreatic islets were evaluated by quantitative computer-assisted morphometric method. The density of beta cells of 28-day-old offspring pancreas significantly reduced from 96.23 ± 5.0 in control group to 71.5 ± 5.3 cells in 10000 mm^2 area of islet in diabetic group ($P < 0.01$). The number of the pancreatic islets of in gestational diabetes (15.25 ± 3.7) significantly reduced in comparison with the controls (8.61 ± 0.7). The density of beta cells of 56-day-old offspring pancreas significantly reduced from 105.33 ± 8.6 in control group to 62.12 ± 5.9 in diabetic group ($P < 0.01$). The number of the pancreatic islets of in gestational diabetes (13.5 ± 0.5) significantly reduced compared to controls (6.75 ± 1.7) ($P < 0.01$). This study revealed that gestational diabetes loss the number of the beta cells in 28 and 56-day-old rat offspring.

KEY WORDS: Gestational diabetes; Beta cell; Pancreas; Offspring; Rat.

INTRODUCTION

Diabetes mellitus as the most common serious metabolic disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins (Gispén & Biessels, 2000; Lebed *et al.*, 2008).

Diabetes mellitus generally classified into type 1 or insulin dependent, type 2 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 experimental studies have reported the adverse effect of gestational diabetes on spinal cord,

cerebellum, hippocampus, ganglionic cell of retina and cortex of cerebrum (Najafdari *et al.*, 2014; Ghafari *et al.*, 2015).

Previous studies have reported the adverse effect of induced diabetes on pancreatic islets (islets of Langerhans) and beta cells of pancreas (Akinola *et al.*, 2010; Akpan *et al.*, 2012). Also, a study reported that the pre-gestational diabetes causes adverse effect on pancreatic islets and beta cells of pancreas (Han *et al.*, 2007).

Indeed, there is no study about the effect of gestational diabetes on endocrine part of pancreas. Therefore, this experimental study was design to assess the effect of gestational diabetes on pancreatic islets and beta cells of pancreas in the postnatal 28 and 56 days of Wistar rats.

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MATERIAL AND METHOD

This experimental study was performed at the Gorgan Faculty of Medicine, 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 the study.

Experimental animals. Wistar rats, weighing 180-220 grams (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 25°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 inject 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.

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 (IP). Blood was sampled from the tail at 1 week after STZ injection. The dams with blood glucose level 120-250 mg/dl known as gestational diabetes (GDM). The pregnancy of dams was terminated physiologically.

Histopathologic and Morphometric techniques. A total of six offspring of gestational diabetic mothers and control mothers at the postnatal day 28 and 56 (P28, P56) were randomly selected and were scarified. For light microscope preparations whole pancreas was fixed in bouin's fluid and paraffin embedded for histological procedure. Histopathologic examination and grading were carried out on chromealum hematoxylin – phloxine (Bancroft & Stevens, 1990) stained sections at 5- μ m thickness with 30- μ m distance were used for morphometric analyses.

The area and number of islets and the density of β -cell of 10000 μ m² area of islet were counted by Olympus BX-51T-32E01 research microscope connected to DP12

Camera with 3.34 million pixel resolution and Olysia Bio software (from: Olympus Optical Co. LTD, Tokyo-Japan).

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).

Statistical analysis. Morphometric data is expressed as the mean \pm SEM and analyzed by the Student's "t" test using SPSS 16.5 software. P < 0.05 was considered significant.

RESULTS

Blood glucose level. In P28 the mean \pm SE of the serum glucose level in control and gestational diabetic offspring were 111.6 \pm 5 and 135.4 \pm 11.2 mg/dl respectively.

In P56 the mean \pm SE of the body weight in control and gestational diabetic offspring were 60.0 \pm 1.6 and 50.5 \pm 6.4 gram, respectively.

Morphometric results. The morphometric findings are depicted in Fig. 1 and Table I.

Beta cells density in the 28-day-old offspring:

In the 28-day-old offspring Mean \pm SE of beta cells in control and gestational diabetic group were analyzed.

The density of beta cells of 28-day-old offspring pancreas significantly reduced from 96.23 \pm 5.0 cells in control group to 71.5 \pm 5.3 cells in 10000 mm² area of islet in diabetic group (P < 0.01).

The number of the pancreatic islets of in GD group (15.25 \pm 3.7) significantly reduced in comparison with the controls (8.61 \pm 0.7) (P < 0.05).

The mean area of the pancreatic islets in the gestational diabetic (11165.0 \pm 2402.7 mm²) non-significantly reduced in comparison with control group (13213.6 \pm 1261.5 mm²).

Beta cells density in the 56-day-old offspring:

In the 56-day-old offspring Mean \pm SE of beta cells in control and gestational diabetic group were analyzed.

The density of beta cells of 28-day-old offspring pancreas significantly reduced from 105.33 \pm 8.6 cells in con-

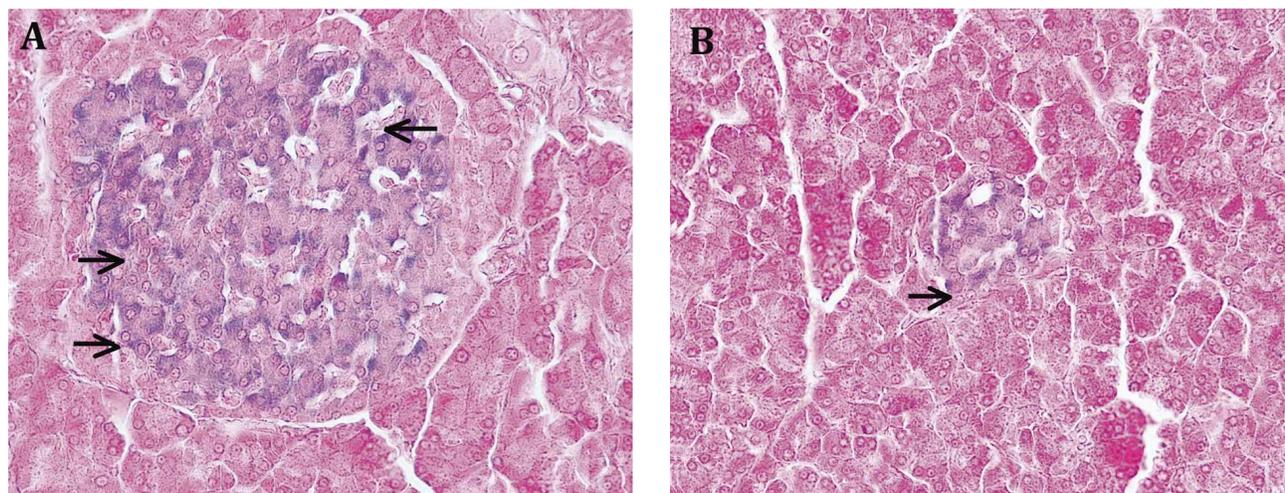


Fig.1. Rat pancreatic section prepared from control (A) and diabetic (B) 56 days Wistar rat offspring. The beta cells are shown blue. The number of beta cells is decreased in diabetic group. Gomori's method staining, (original magnification X400, the arrows show the beta cell).

Table I. The quantitative characteristics of pancreatic islets in postnatal day 28 and 56 of gestational diabetes and control offspring Wistar rat

	P28		P56	
	Control	Gestational diabetic	Control	Gestational diabetic
Number of beta cells/10000	96.23±4.9	71.56±5.3*	105.33±8.6	62.12±5.9*
pancreatic islets area	13213.6±1261.5	11165.0±2402.7	14026.9±1575.6	11019.0±3222.3
Number of islet	15.25±3.7	8.61±0.7*	13.5±0.5	6.75±1.7*

Results are expressed as Mean±SE of the mean, * P <0.05 versus control, n=6

control group to 62.12±5.9 cells in 10000 mm² area of islet in diabetic group (P <0.05).

The number of the pancreatic islets of in GD group (13.5±0.5) significantly reduced in comparison with the controls (6.75±1.7) (P <0.05).

The mean area of the pancreatic islets in the gestational diabetic (11019.0±3222.3 mm²) non-significantly reduced in comparison with control group (14026.9±1575.6 mm²).

DISCUSSION

The present study demonstrated that gestational diabetes produces a significant reduction in the density of beta cells and number of the pancreatic islets of pancreas in the postnatal day 28 and 56 of Wistar rats.

Previous studies have reported the adverse effect of induced diabetes on pancreatic islets and beta cells of

pancreas (Akinola *et al.*; Akpan *et al.*). Also, a study reported that the pre-gestational diabetes cause adverse effect on pancreatic islets and beta cells of pancreas (Han *et al.*).

Also, Akinola *et al.*, reported that the density of beta cells significantly reduces in diabetic rates in comparison with controls. According to his opinion hyperglycemia disrupted the cells of pancreatic islets.

Indeed, Akpan *et al.*, study showed that hyperglycemia in diabetic rats induced morphological alterations in pancreatic islets cells including dark necrotic area in pancreatic islets, cellular edema and inflammation.

Furthermore, Han *et al.*, reported that the hyperglycemia in diabetic rats induced morphological alterations in the size and number of pancreatic islets of offspring.

The two main forms of diabetes including type I and type II diabetes are characterized by progressive β-cell failure. In type I, β-cell failure typically caused by an autoimmune response against the β-cells, inducing

progressive β -cell death while in the type II, comprising different degrees of β -cell failure relative to varying degrees of insulin resistance (Cnop *et al.*, 2005).

In our study, b-cell number is reduced by 20–40 % at the 28 and 56 days offspring's after induction of gestational diabetes, respectively.

In type 1 diabetes, 70–80 % of β -cell mass is reduced at the time of diagnosis, because of β -cell necrosis, it was suggested that β -cell loss occurs slowly over years (Kloppel *et al.*, 1985).

It was shown that β -cell apoptosis causes a gradual β -cell depletion in rodent models of type 1 diabetes (Eizirik & Mandrup-Poulsen, 2001). In type 2 diabetic subjects, initial pathological studies suggested a β -cell loss of 25–50 % (Kloppel *et al.*)

Significant reduction in β -cell mass and a threefold increase in β -cell apoptosis (Butler *et al.*, 2003), suggested that β -cell mass is reduced in type 2 diabetes, secondary to increased rates of b-cell apoptosis (Kahn, 2003).

β -cell apoptosis may be a common feature of type 1 and type 2 diabetes. Several studies suggest that both forms of diabetes are characterized by intra-islet expression of inflammatory mediators such as cytokine interleukin [IL]-1 β), triggering a final common pathway of β -cell apoptosis, progressive β -cell loss, and diabetes (Donath *et al.*, 2003).

In the type 1 diabetes, apoptosis, the main cause of β -cell death, is a highly regulated process, activated and/or modified by extracellular signals, intracellular ATP levels, phosphorylation cascades, and expression of pro- and anti-apoptotic genes (Eizirik & Mandrup-Poulsen). Cytokines induce stress response genes that are either protective or deleterious for β -cell survival. In extensive microarray experiments (Rasschaert *et al.*, 2003), several genes and expressed sequence tags are identified that are up- or down regulated in purified rat β -cells or insulin-producing cells after 1–24 h of exposure to IL-1 β and/or IFN- γ .

IFN- γ binds to cell surface receptors and activates the tyrosine kinases JAK1 and JAK2. These kinases phosphorylate the transcription factor STAT-1, which dimerizes and translocates to the nucleus to bind to γ -activated sites of diverse genes (Eizirik & Mandrup-Poulsen). STAT-1 mediates the potentiating effect of IFN- γ on IL-1 β -induced iNOS expression (Darville & Eizirik, 1998). Activation of JAK/STAT signaling may lead to cell death (Cnop *et al.*).

Overlay, IL-1 β -induced NF- κ B activation plays a key role in controlling multiple and distinct gene regulatory networks, which affect the β -cell-differentiated state and ER Ca²⁺ homeostasis, activate immune cells and directly contribute to β -cell apoptosis (Cnop *et al.*).

Also, activation of the stress-activated protein kinases c-Jun NH₂-terminal kinase (JNK), p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK), triggering of ER stress and the release of death signals from the mitochondria are probable mechanisms in β -cell death (Cnop *et al.*). Also, changes in ER Ca²⁺ concentrations induced disruption of ER homeostasis caused ER stress response (Schroder & Kaufman, 2005). In case of prolonged and severe ER stress, the apoptosis program is activated and executed by the transcription factor CHOP, the MAPK JNK, and caspase-12 (Schroder & Kaufman).

Mitochondria not only have key role for β -cell survival and function, but also, play an important role in triggering apoptosis (Newmeyer & Ferguson-Miller, 2003). Members of the Bcl-2 protein family regulate the mitochondrial response to pro-apoptotic signals (Newmeyer & Ferguson-Miller), preventing release of mitochondrial proteins including cytochrome c, which, activate caspase-9 and -3 and execute cell death (Friedlander, 2003). Cytokines disrupt the mitochondrial membrane potential in RINm5F cells, which is prevented by overexpression of the anti-apoptotic protein Bcl-2 (Barbu *et al.*, 2002). Overexpression of Bcl-2 against cytokine-induced cell death, but does not prevent spontaneous diabetes in non-obese diabetic (NOD) mice (Allison *et al.*, 2000). This suggests that other mechanisms, bypassing Bcl-2, induce β -cell death *in vivo* and/or that Bcl-2-regulated mitochondrial events and caspase activation are late steps in the apoptosis process, occurring when the cell fate has already been decided.

In type 2 diabetes, when β -cells chronically exposed to high glucose, several alterations of their phenotype, including changes in glucose stimulus-secretion coupling, gene expression, cell survival, and cell growth were observed (Rhodes, 2005). These alterations could result from cytokine, oxidative stress or ER stress-induced changes in gene expression and cell survival (Schroder & Kaufman) or from functional changes that are not directly related to β -cell apoptosis, such as accumulation of glycogen.

Several genes expressed at low levels in normal β -cells are induced by hyperglycemia, including hexokinase 1, lactate dehydrogenase and glucose-6 phosphatase. In addition, pro- and anti-apoptotic factors, antioxidant enzymes, and some transcription factors are upregulated (Weir *et al.*, 2001). c-Myc, A20, and heme-oxygenase 1, are

induced by hyperglycemia and cytokines, suggesting that both conditions share some common mechanisms to alter the β -cell phenotype.

In type 2 diabetes models, β -cell losing occurs mainly by necrosis (Jorns *et al.*, 2002). The necrotic cells are removed by scavenger macrophages, which, at variance from the type 1 diabetes situation, are not activated and do not express the pro-inflammatory cytokines IL-1 β , IFN- γ , or TNF- α (Cnop *et al.*).

Han *et al.* study in animal model showed that gestational diabetes may be similar to type 2 diabetes models and by effect on carbohydrate metabolism and reducing some enzymes which involved in carbohydrate metabolism cause the type 2 diabetes models in rat offspring.

CONCLUSION

This study revealed that gestational diabetes produces a significant reduction in the density of beta cells and number of the pancreatic islets of pancreas in the postnatal day 28 and 56 of Wistar rats. Further studies are required for determining the exact mechanism of beta cell loss of offspring born from gestational diabetes in animal and human.

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RESUMEN: Estudios previos han mostrado los efectos adversos de la diabetes gestacional en la densidad neuronal del hipocampo y de la médula espinal en modelos animales. Este estudio se llevó a cabo para determinar el efecto de la diabetes gestacional en las células beta del páncreas de rata en vida postnatal temprana. En este estudio experimental, 10 ratas fueron asignadas al azar a los grupos control y diabético en el día 1 de gestación. Cinco ratas del grupo diabético recibieron 40 mg/kg/BW de estreptozotocina (intraperitonealmente), mientras que los animales del grupo control recibieron solución salina normal. Seis de los descendientes, de 28 y 56 días de edad, de cada grupo, diabetes mellitus gestacional y control, se escarificaron al azar y se tomaron secciones del páncreas, que se tiñeron usando el método de Gomorra. La densidad de las células beta y el número y área de islotes pancreáticos fueron evaluados a través de método cuantitativo asistido por computadora morfométrica. La densidad de células beta del páncreas en las crías de 28 días disminuyó significativamente de $96,23 \pm 5,0$ en el grupo de control a $71,5 \pm 5,3$ células en el grupo diabético, en 10000 mm^2 de área de islote ($P < 0,01$). El número de islotes pancreáticos de la diabetes gestacional ($15,25 \pm 3,7$) se redujo significativamente en comparación con los controles ($8,61 \pm 0,7$). La densidad de células beta del páncreas en las crías de 56 días de edad se redujo de $105,33 \pm 8,6$ en el grupo de control a $62,12 \pm 5,9$ en el grupo diabético ($P < 0,01$). El número de islotes pancreáticos en el grupo de diabetes gestacional ($13,5 \pm 0,5$) se redujo significativamente en comparación con los controles ($6,75 \pm 1,7$) ($P < 0,01$). Este estudio reveló que la diabetes gestacional provoca una pérdida en el número de células beta en crías de ratas de 28 y 56 días de edad.

PALABRAS CLAVE: Diabetes gestacional; Célula beta; Páncreas; Crías; Rata.

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