Maternal Diabetes Mellitus Disturbs Histological Architecture and Integrity of Liver and Kidney in Rat Offspring

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Abstract

This study was designed to investigate the effect of maternal diabetes in rat dams on histological architecture and integrity of liver and kidney of their offspring. Diabetes mellitus was induced in rat females by single intraperitoneal injection of streptozotocin at dose level of 45 mg/kg b. w. Proesterous normal and diabetic females were left for one night to copulate with the normal males (2 females with one male) and the resulting pregnant rat dams were allocated into two main groups, normal control group and streptozotocin-induced diabetic group. After birth, the surviving offspring were subjected to histological examination of liver and kidney immediately after delivery and at the end of the 1\textsuperscript{st} and 2\textsuperscript{nd} postnatal weeks. The liver and kidney weights relative to body weight, determined at the same periods, were significantly increased in offspring of diabetic dams only at the end of the 2\textsuperscript{nd} week as compared to those of the corresponding offsprings of normal rat dams. The liver of diabetic rat offspring after birth showed abnormal histological architecture since the hepatic strands are less organized and many hepatocytes with pyknotic nuclei. After one week of birth, the liver section revealed severe widening of the central vein, albuminous material accumulation with focal necrotic areas and degenerated hepatocytes associated with abnormal distribution of condensed cytoplasm and of karyoplasm. After two weeks of birth, the liver appeared even more deteriorated than before. The central vein was congested with blood. The hepatocytes were hydropically degenerated and their nuclei became pyknotic. The kidney of diabetic rat offspring, on the other hand, showed mild occlusion of the renal tubules with albuminous material accumulation at birth. The glomeruli were smaller and more condensed. After the 1\textsuperscript{st} postnatal week, edema and mononuclear leucocytic infiltration were observed and many tubules still suffer from occlusion. After two weeks of birth, the kidney section illustrated severe degenerative changes of renal tubules with intratubular fibroblastic proliferation. In conclusion, the maternal diabetes during the period of gestation in rat dams deleteriously affects histological architecture and integrity of the offspring liver and kidney.

Key Words: Maternal diabetes mellitus, offspring, liver, kidney

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of DM is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood...
vessels as well as liver (Ahmed, 2001; American Diabetes Association [ADA], 2014).

Pregnancies under diabetic conditions are complicated by a number of factors possibly leading to abnormalities during fetal and embryonic development preterm delivery (Melamed et al., 2008). Miscarriage and stillbirth are more common in pregnant women with diabetes; the effect maternal diabetes on neonates could also not be neglected (The American College of Obstetricians and Gynecologists, 2009).

Maternal diabetes in the rat can either increase or decrease foetal growth depending on the severity of the hyperglycaemia. Fetal complications include macrosomia, neonatal hypoglycemia or hyperglycemia, perinatal mortality, congenital malformation, hyperbiliruinemia, polycythemia, hypocalcemia, and respiratory distress syndrome (Metzger et al., 1998; Kjos and Buchanan, 1999; Sheffield et al., 2002; Aref et al., 2013).

A variety of diabetic animal models during pregnancy are used to assess long-term effects on the offspring. A concern of studies using STZ during pregnancy is the possibility that the toxin might cross the placenta and be directly harmful to the fetal pancreas and other fetal tissues, thus, making any analysis of the long term effects of hyperglycemia in utero difficult (Ayan et al., 1995). Thus, the pre-existing streptozotocin-induced diabetes mellitus in pregnant rats was most commonly used by several authors (Han et al., 2007; Aref et al., 2013).

The effect of maternal diabetes on insulin secretion, β-cell function and metabolic status in offspring was previously studied in our previous publication (Aref et al., 2013). However, the effects of maternal diabetes on histological architecture and integrity of liver and kidney in offspring are scarcely investigated by previous publications. Thus, study aims to assess the effect of pre-existing STZ-induced diabetes during pregnancy of rat dams on liver and kidney architecture and integrity on the offspring at different post-natal periods.

**MATERIALS AND METHODS**

**Experimental animals**

Experiments were carried out on 65 albino rats (Rattus norvegicus), 55 mature virgin females weighing about 170–200 g, and 10 mature males 190–220 g. The animals were obtained from the Animal House, Faculty of Medicine, Assiut University, Egypt. All animal procedures are in accordance with the general guidelines of animal care and the recommendations of the Canadian Committee of Canadian Council on Animal Care (1993). All efforts were made to minimize the number of animals used and their suffering. Adult rats were kept under observation for 2 weeks before experimentation to exclude any intercurrent infection and to acclimatize the animals to the new conditions. The selected animals were marked, housed in stainless steel cages with separate bottom, and kept at a temperature of 23 ± 2°C, with good ventilation and a relative humidity of 50 ± 5%. The animals were exposed to constant light/dark periods of 12 hours (hr) each (light on at 06:00 hr) and fed on standard rodent pellet diet as well as some vegetables as a source of vitamins. For drinking tap, water was provided ad libitum.

**Induction of diabetes mellitus**

Diabetes mellitus was experimentally induced in female virgin animals fasted for 16 hours by intraperitoneal injection of 45mg/kg b.wt. streptozotocin (Sigma-Aldrich Chemie GmbH, Germany) dissolved in citrate buffer (pH 4.5) (Ahmed et al., 2007; Abdel-Reheim et al., 2007). Ten days after streptozotocin injection, rats were deprived of food and water overnight and blood samples were obtained from lateral tail vein after two hours of oral glucose loading (3 g/kg b.w.) Serum glucose level was measured for each female rat. Rats with serum glucose level higher than 180mg/dl were considered as diabetic and were included in the experiment, while others were excluded.

**Mating and fertilization**

To determine the estrus cycle, the vaginal smear of each virgin female was examined daily. Three types of cells, leukocytes and epithelial and cornified cells were observed in photomicrographs of unstained vaginal smear. As reported by Marcondes et al. (2002), the proportion of the three types of cells was used for the determination of the estrous cycle phases. A proestrous smear consists of a predominance of nucleated epithelial cells; an estrous smear primarily consists of anucleated cornified cells; a metestrus smear consists of the same proportion among leukocytes, cornified, and nucleated epithelial cells; a diestrus smear primarily consists of a predominance of leukocytes.

Proesterous normal and diabetic females were left for one night to copulate with the normal males (2 females with one male). Early next morning (before 7 am), copulation was checked by examining the outer surface of the vagina for the presence of a vaginal plug formed by coagulation of semen (white clotting, sperm clot). When such a grayish-white clot blocking the mouth of vagina was detected, this day was considered as the first day of gestation.

**Animal grouping and tissue sampling**

Adult female pregnant rat dams were divided into two
groups. Group 1 consists of normal female animals. It is regarded as control. Group 2 is composed of STZ-induced diabetic females. After gestation and delivery, the offspring of normal pregnant female dams were kept in separate cages from those of the diabetic pregnant female dams. At birth and at the end of the 1st and 2nd post-natal weeks, the offspring were sacrificed and dissected. Liver and kidney were rapidly excised and weighed. Then, kidney and pieces of liver were fixed in 10% neutral buffered formalin pending processing to prepare stained histological sections.

**Determination of relative liver and kidney weights**

The relative weights of liver and kidney of offspring of normal and diabetic females immediately at birth and the end of the 1st and 2nd post-natal weeks were calculated from the formula: relative organ weight = organ weight/body weight x 100.

**Microscopic examination of liver and kidney**

At specific time intervals (zero time, after one and 2 weeks), offspring of both normal and diabetic dams were sacrificed and liver and kidney were immediately excised. Pieces of liver and kidney were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut serially at 5 µm thickness and stained with hematoxylin and eosin. Before staining the sections were de-waxed and hydrated. After staining the sections were dehydrated in alcohol, cleared in xylene mounted in Canada balsam. Micrographs were taken using 40x light microscope.

**Statistical Analysis**

The data are analyzed by one-way analysis of variance (ANOVA) using PC-STAT, University of Georgia, followed by LSD analysis to discern the main effects and to compare various groups with each other (Roa et al., 1985). F-probability for each variable expresses the general effect between groups. A two-way analysis of variance was also applied to evaluate the effect of time, diabetes, and their interaction during the experimental periods. The data are presented as mean ± standard error (SE) and values of p > 0.05 are considered statistically nonsignificant, while those of p < 0.05, p < 0.01, and p < 0.001 are considered statistically significant, highly significant, and very highly significant, respectively.

**RESULTS**

**Effect on relative liver weight**

The data of relative liver weight of offspring of normal and diabetic females at birth and at the end of the 1st and 2nd post-natal weeks are represented in table 1. At birth, the relative liver weight of normal offspring showed a mean of 4.75± 0.10. As the post-natal period extended to 1 week, the mean relative liver weight significantly (p<0.01) decreased to reach a value of 2.80 ± 0.20. After 2 weeks, the mean liver relative was increased again and reached a value of 2.88 ± 0.11 which was not significant when it was compared with that at the 1st week.

The liver relative weight of diabetic rat neonates was at birth 4.70±0.10 and did not differ significantly (p>0.05) from that of control group. The difference was – 1.05%. After 1 week, the mean liver relative weight was decreased to a value of 2.71± 0.04 which was still insignificantly lower (p>0.05) as compared to control; percentage change was -3.21%. After 2 weeks, the relative liver weight of diabetic rat offspring was increased again and was significantly (p<0.05) greater than that of control. It reached a mean value 3.20± 0.10. The difference between normal and diabetic offspring was +11.11 %.

With regard to two-way ANOVA, the effect of time, diabetes and time-diabetes interaction relative liver weight was significant with probabilities p<0.001, p<0.01 and p<0.05 respectively. Data are given as mean± S.E.; Means with the same superscript symbol are not significantly different; 0 week = at birth, 1 week = end of the 1st post-natal week and 2 weeks = end of the 2nd post-natal week; n =number of observations; % Difference = difference between normal and diabetic offspring (normal = 100%).

**Effect on relative kidney weight**

The relative weight of kidney of offspring of normal and diabetic females immediately at birth and the end of the 1st and 2nd post-natal weeks is represented in table 2. At birth, the relative weight of kidneys (weight of 2 kidneys) of normal rat offspring showed a value of 1.01±0.04. After 1 week, the relative kidney weight significantly (p<0.05) increased to a value of 1.28±0.02. After 2 weeks, the relative kidney weight was decreased again to 1.12±0.06 which was significant (p<0.05) as compared with the value at the 1st week.

The relative kidney weight of diabetic offspring at birth was slightly, but insignificantly higher (p>0.05) than that of the normal control; the value accounted was 1.05±0.04 and differed by + 3.96% from normal control. After 1 week, the relative organ weight significantly (p<0.05) increased to 1.31±0.02 and remained constant until the end of the experimental period. The difference from control was + 2.34 % and 16.96 % the end of the 1st and 2nd weeks respectively. The effect of maternal diabetes was insignificant (p>0.05) at birth and at the end of the 1st week while it was significant (p<0.05) at the end of the 2nd week as compared to the corresponding normal control.
Table 1. Relative weight of liver of offspring of normal and diabetic females at different experimental periods.

<table>
<thead>
<tr>
<th>Periods</th>
<th>Normal offspring</th>
<th>Diabetic offspring</th>
<th>% Difference</th>
<th>F-probability</th>
<th>LSD at 5% level</th>
<th>LSD at 1% level</th>
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<tr>
<td>0 week</td>
<td>4.75 ± 0.10&lt;sup&gt;a&lt;/sup&gt; (n=15)</td>
<td>4.70 ± 0.10&lt;sup&gt;a&lt;/sup&gt; (n=22)</td>
<td>-1.05%</td>
<td>p &lt; 0.001</td>
<td>0.33</td>
<td>0.45</td>
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<td>1 week</td>
<td>2.80 ± 0.20&lt;sup&gt;b&lt;/sup&gt; (n=20)</td>
<td>2.71 ± 0.04&lt;sup&gt;b&lt;/sup&gt; (n=19)</td>
<td>-3.21%</td>
<td></td>
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<tr>
<td>2 weeks</td>
<td>2.88 ± 0.11&lt;sup&gt;c&lt;/sup&gt; (n=17)</td>
<td>3.20 ± 0.10&lt;sup&gt;c&lt;/sup&gt; (n=20)</td>
<td>+11.11%</td>
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Table 2. Relative weight of kidneys of offspring of normal and diabetic females at different experimental periods

<table>
<thead>
<tr>
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<th>Diabetic offspring</th>
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<td>+11.11%</td>
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With regard to the two-way ANOVA, the effect time, diabetes and time-diabetes interaction significant (p<0.05), very highly significant (p<0.001) and insignificant (p>0.05) respectively.

Data are given as mean± S.E.; Means with the same superscript symbol are not significantly different; 0 week = at birth, 1 week = end of the 1<sup>st</sup> post-natal week and 2 weeks = end of the 2<sup>nd</sup> post-natal week; n =number of observations; % Difference = difference between normal and diabetic offspring (normal = 100 %).

Effect of maternal diabetes on liver histological changes of the offspring

The haematoxylin and eosin stained liver sections of control rat offspring at birth revealed normal architecture consisting of numerous hepatic lobules (classical lobules) and connective tissue septa in between (figure 1A). These septa are conspicuous. The liver sinusoids appear to be continuous from one lobule to another. Portal areas containing the interlobular branches of portal veins (PV), hepatic arteries (a) and bile ducts (bd) are visible around the peripheries of different lobules. In the center of each of these hepatic lobules, there is a central vein (CV). Radiating from the central vein towards the periphery of the lobules are strands of hepatic cells and trabeculae (T). Located between the strands of hepatocytes are hepatic sinusoids (s) and Kupffer cells (kc).

During the next two time intervals the architecture of the liver became more organized and by the end of the 3<sup>rd</sup> postnatal week, numerous Kupffer cells were observed (figures 2A, 3A).

The liver of diabetic rat offspring after birth showed abnormal liver architecture. Hepatic strands are less organized and many hepatocytes with pyknotic nuclei
Figures 1A and 1B. Normal rat offspring after birth showing normal liver architecture (figure 1A). Portal vein (pv), bile ductile (bd) and hepatic artery (ha) were observed. Diabetic rat offspring just after birth illustrating many hepatocytes (h) with pyknotic nuclei (pk) indicated by arrows (↓) (figure 1B). (X400)
Figures 2A and 2B. Normal rat offspring after one week of birth showing higher organization of liver architecture with sinusoids (s), central vein (cv), hepatic strand (↓) (figure 2A). Diabetic rat offspring after one week of birth illustrating severe widening of the central vein (wcv) and albuminous material accumulation (*). Focal necrotic areas (nc) were also noticed (figure 2B). (X 400)
Figures 3A and 3B. Normal rat offspring after two weeks of birth illustrating hepatic strands (↓) arising from the central vein (cv) and separated by sinusoids (s). Numerous Kupffer (k) cells were observed in the sinusoids (figure 3A). Diabetic rat offspring after two weeks of birth showing congestion of the central vein (ccv) and hydropic degeneration of the hepatocytes (↓) (figure 3B). (X 400)

Effect of maternal diabetes on kidney histological changes of the offspring

Kidney section of control rat offspring at birth revealed normal histological structure of the tissue (figure 4A). The kidney is divided into an outer region, the cortex and an inner one, the medulla that sends medullar rays. The cortex contains glomeruli, proximal tubules and distal tubules. The medulla contains collecting tubules, thin segments of loop of Henle and straight of distal tubules. After one week of birth, the kidney section shows normal glomeruli, proximal tubules and distal tubules and mild (pk) were observed (figure 1B).

After one week of birth, the liver section revealed severe widening of the central vein and albuminous material accumulation with focal necrotic areas. Hepatocytes were degenerated showing abnormal distribution of condensed cytoplasm and of karyoplasms (figure 2B).

After two weeks of birth, the liver appeared even more deteriorated than before. The central vein was congested with blood. The hepatocytes were hydropically degenerated and their nuclei became pyknotic (figure 3B).
Figures 4A and 4B. Normal rat offspring after birth showing normal malpighian corpuscles with normal glomerulus (g) surrounded by a Bowman’s capsule (↓). A proximal tubule (px), a distal (d) tubule and urinary (u) space were also noticed (figure 4A). Diabetic rat offspring after birth illustrating mild occlusion of the renal tubules with albuminous material accumulation (↓). Glomeruli are smaller and more condensed (Figure 4B). (X 400).
Figures 5A and 5B. Normal rat offspring after one week of birth showing mild oedema (ο) (figure 5A). Diabetic rat offspring after one week of birth demonstrating oedema (ο) and mononuclear leucocytic infiltration (↓). Many tubules still suffer from occlusion (*) (figure 5B). (X 400)
odema (figure 5A). After two weeks, the section shows normal histological architecture (figure 6A).

The kidney of diabetic rat offspring showed at birth mild occlusion of the renal tubules with albuminuous material accumulation. The glomeruli were smaller and more condensed (figure 4B). After the 1st postnatal week, oedema and mononuclear leucocytic infiltration were observed and many tubules still suffer from occlusion (figure 5B). After two weeks of birth, the kidney section illustrated severe degenerative changes of renal tubules with intratubular fibroblastic proliferation (figure 6B).

**DISCUSSION**

The increase in type 2 diabetes in women at reproductive age and the cross-generation of the intrauterine programming of type 2 diabetes are the bases for the growing interest in the use of experimental diabetic models in order to gain insight into the mechanisms of induction of developmental alterations in maternal diabetes. Using the appropriate animal model, several important aspects of human diabetic pregnancies such as the increased rates of spontaneous abortions, malformations, feto-placental impairments, and offspring diseases in later life can be approached (Van Assche et al., 1991a and b; Jawerbaum and White, 2010).

Therefore, the present study tends to investigate the effect of maternal diabetes on the relative organ weights as well as the histological architecture and integrity of two important organs of the body, liver and kidney of the offspring.

Female rats with pre-existing diabetes mellitus were mated with healthy, non-diabetic males and the observed outcome were compared to offspring of non-diabetic
Diabetes was experimentally induced by intraperitoneal injection of 45 mg/kg b.wt. streptozotocin which was suitable to produce elevation in the serum glucose level higher than 18 mg/dl after 2-hours oral glucose administration to overnight fasted female rats.

Several reports have been published on the effect of streptozotocin on the glycemic state of different animal species (Gunnarson et al., 1974; Yamamoto et al., 1981; Uchigata et al., 1983; Okamoto, 1984; Abdel-Moneim et al., 1997, 1999). Most of these studies indicated that the substance has a cytotoxic effect on beta-cells of the pancreatic islets and can induce chronic or permanent diabetes in animals. Moreover, the possible mechanism for beta-cells destruction by streptozotocin includes (a) generation of oxygen free radicals and alteration of endogenous scavengers of these reactive species; (b) breakage of DNA and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting nicotinamide adenine dinucleotide in beta-cells; (c) inhibition of active transport and calmodulin-activated proteinase activity; and (d) induced alteration of islet mitochondrial function (Gaulton et al., 1985; Yoon, 1990; Marles and Farnsworth, 1995; Pusztai et al., 1996).

Animals with chemically induced diabetes have been used to study either insulin dependent diabetes mellitus (IDDM) (Mathe, 1995; O Brien et al., 1996; Ulicna et al., 1996; Ohno et al., 1998; Abdel-Moneim et al., 1999) or non-insulin dependent diabetes mellitus (NIDDM) (Ostenson et al., 1989; Ali et al., 1993; Masiello et al., 1998). Sensitivity to streptozotocin varies with species, strain, sex, age and nutritional state (Bailey and Flatt, 1991), but it was clearly demonstrated by Ulicna et al. (1996) that IDDM can be induced in adult rats by a single dose of streptozotocin (45 mg/Kg b. w.).

In the present study, the liver and kidney weights relative to body weight were significantly increased in the offspring of streptozotocin-induced diabetic rat dams than those of normal rat dams at the end of the 2nd post-natal age. These results are in agreement with Lee (2009) who reported that infants born to diabetic mothers may have large organs. Other investigators elucidated that growth occurs preferentially in insulin-sensitive tissues such as adipose tissue, heart and liver (Kehl et al., 1996; Landon et al., 1996).

In the current study, the liver of diabetic rat offspring after birth showed abnormal liver architecture since the hepatic strands are less organized and many hepatocytes have pyknotic nuclei. As the post-natal period extended to 1 week, the liver of offspring of diabetic dams exhibited severe widening of the central vein, albuminous material accumulation with focal necrotic areas and hepatocytes degenerative with abnormal distribution of condensed cytoplasm and of karyoplasts. At the end of the 2nd post-natal week, the liver appeared even more deteriorated than before, the central vein was congested with blood and the hepatocytes were hydropically degenerated and their nuclei became pyknotic. These liver histological changes may be secondary to the persistent hyperglycemia at birth and at the 1st and 2nd post-natal weeks due to impaired β-cell function and insulin resistance as indicated in our previous study (Aref et al., 2013). In accordance with the present study, Weintrob et al. (1996) reported hyperbilirubinemia, an indicator of impaired liver function, as one of short-term neonatal complications in offspring of diabetic mothers. Ahmed (2001) found that liver of STZ-induced diabetic rats exhibited hyperemic central veins, sinusoids and portal veins associated with diffuse proliferation of kupffer cells, focal mononuclear leucocytic infiltration and spotty necrosis in the hepatocytes.

Similarly to the liver, the kidney of diabetic rat offspring exhibited many histological perturbations including occlusion of the renal tubules with albuminous material accumulation and smaller and more condensed glomeruli at birth, edema, mononuclear leucocytic infiltration and occlusion of many renal tubules at the 2nd post-natal week, as well as severe degenerative changes of renal tubules with intratubular fibroblastic proliferation at the 3rd post-natal week. These results are in concurrence with Ahmed (2001) who revealed that the kidney of STZ-induced diabetic rats showed fibroblastic proliferation between renal tubules and degenerative changes in the endothelial cells lining tubules. In our opinion, the deteriorated effects of maternal diabetes on the kidney may be attributed to the persistent hyperglycemia in the neonates as indicated by our previous publication (Aref et al., 2013). Another explanation stated by Chen et al. (2010) is that offspring of diabetic mothers developed hypertension, microalbuminuria, and glucose intolerance which in turn have deteriorated effects on the kidney.

CONCLUSION

In conclusion, the maternal diabetes mellitus in rat dams have deleterious effects on the liver and kidney of offspring.

REFERENCES


