The effects of maternal diabetes on the glomerular and human volume and tubular changes in newborn rats

Maryam Tehranipour* , Saiede Moosavi

Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran. Corresponding Author: maryam_tehranipour@mshdiau.ac.ir

Abstract: Maternal diabetes is associated with an increased risk of several complications in the offspring. In present study, we examined the effects of maternal diabetes on the glomerular and human volume and tubular changes in newborns rats. At 7th day of pregnancy hyperglycemia was induced by a single injection (i.p.) of streptozotocin (55 mg kg\(^{-1}\)). Control animals were given an equal volume of citrate buffer. After parturition 1 pup were randomly selected from each litter, their kidney dissected, fixed in 10% formalin, sectioned in 7 \(\mu\)m thicknesses and stained by H.E. By applying stereological techniques and systematic random sampling scheme the glomeroli, human, proximal and distal diameters changes were estimated. In comparison with controls, statistical analysis showed significant increases (p<0.05) in the glomeroli and human volumes. The total length of capillaries showed significant increases (p<0.01) in newborns from diabetic mothers. But there were not significant different in proximal and distal diameters. In conclusion it seems that, maternal diabetes induce angiogenesis. The present study is high risk for renal dysfunction in newborns from diabetic mothers. [Maryam Tehranipour, Saiede Moosavi, The effects of maternal diabetes on the glomerular and human volume and tubular changes in newborn rats Life Sci J 2013;10(1s):470-473](http://www.lifesciencesite.com) (ISSN:1097-8135).

Key words: maternal diabetes, kidney, angiogenesis.

Introduction

Offspring of mothers with diabetes mellitus remain at risk for fetal hyperinsulinenia, consequent increase fetal adiposity and often excess fetal size (macrosomia) which increases the likelihood birth trauma and operative delivery. In this neonates probability of maternal disorders is about 3-8%, such as numerous complications in nervous system, kidney, eye and vascular system [1]. In addition, many studies indicate that maternal metabolic abnormalities seen in gestational and preexisting diabetes have long term consequences on weight and pancreatic function and neurological development of the offspring [2]. Extensive experimental and clinical evidence indicates that metabolic disturbances in the mother contribute to virtual all the adverse effects of Diabetes Mellitus on the offspring [3]. Diseases such as maternal diabetes create an adverse in utero environment that may impair the process of embryogenesis, thus predisposing infants of low birth weight (LBW) to subsequent increased risk for future disease [4]. The developing kidney seems particularly sensitive to a high-glucose milieu, exposure to which may result in congenital renal malformations, such as renal agenesis, dysplasia, or hypoplasia [5]. Intrauterine growth restriction (IUGR) leads to a reduction in nephron endowment at birth and is linked to renal dysfunction in adulthood [6]. We know that maternal diabetes programs the offspring to develop hypertension and kidney injury in adulthood [7]. In addition, angiogenesis affects kidney development [8,9]. In this respect, moderate hyperglycemia induces a defect in angiogenesis as reported in experimental conditions [10]. We established that maternal diabetes can change the choroidal plexus volume and increase the length of choroidal capillary in neonates’ brain [11]. It has been clear that hyperglycemia in embryonic time can effect on angiogenesis process in microvascular system in brain, but whether it is effected on glomerular plexus in kidney or not? We hypothesized that the effects of hyperglycemia on kidney development Changes in glomerular volume, play an important role in the initiation and progression of various glomerulopathies. This study investigated the effects of maternal diabetes on the glomerular and human volume and tubular changes in newborns.

Materials and methods

All experiment was conducted in faculty of science, Islamic Azad University – Mashhad Branch, Iran (2012-2013). All chemical used in this study were purchased from Sigma (UK).

Animal subjects:

Wistar rats were used for this study. The study was approved by the committee of our institute. Young adult female rats (approximately 250 gr) were maintained at 22\(c\) with 12-h periods of light and darkness. They were mated with normal males and the morning of appearance of vaginal plug was considered day 0 of gestation. At 7 days of gestation (dg), diabetes was induced by a single injection (i.p.) of streptozotocin (55mg/kg) dissolved in sterile phosphate buffered saline [12]. Control group received only buffer. Then animals were housed under standard condition and received food and water. Induction of diabetes was confirmed by blood glucose level (glycemia>400). After birth one pups of
each mother was selected randomly for stereological analysis.

**Blood assays**

Blood samples were collected from mothers after delivery of the pups and the levels of glucose, uric acid, urea, Triglycerides and Cholesterol were measured by auto analyser.

**Sampling**

Under the pentobarbital anesthesia the newborns’ kidney was rapidly removed and fixed in 10% paraformaldehyde. For histological evaluation samples were placed in same fixative overnight and were embedded in paraffin. Serial cross sections were cut and stained with hematoxylin and eosin. The glomerular plexus and buman volumes were estimated by the unbiased disector/Cavalieri approach [13]. This method requires only a serial section through each glomerulus and is therefore well suited to clinical biopsy specimens with a limited number of glomerular profiles. The length of capillaries was gained by counting described by Gunderson et al. (1988)[14].

**Statistical analysis:**

Student's t test was used for comparison when only two groups were analyzed. Statistical significance was chosen as p<0.05. All results are reported as Mean ± SEM.

**Table.1: Concentrations of different metabolites in control and diabetic rat serum.**

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Cratinin</th>
<th>Uric acid</th>
<th>Urea</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>105 ± 5</td>
<td>0/46 ±0/03</td>
<td>1/91 ± 0/1</td>
<td>18 ± 1/6</td>
<td>119/8 ± 22</td>
<td>47/13 ± 2/7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>450± 18</td>
<td>0/53 ±0/06</td>
<td>5/48 ± 0/7</td>
<td>28 ± 2/6</td>
<td>216/9 ± 49</td>
<td>87/38 ± 7/6</td>
</tr>
</tbody>
</table>

Values are means± SEM, n=6. p<0.05 indicates significant difference from control determined by Student s t test.

**Results**

We investigated the maternal diabetes effects on glomerular plexus, buman volumes and tubular changes in newborn rats. Experimental diabetes was induced by streptozotocin on female rats. In order to confirm of induced diabetes, blood glucose was tested 48 hours after first injection and there after each three days. Diabetes was assessed in this study by monitoring the blood glucose levels in both PBS and STZ injected rats (Table 1). There was a significant increase (p<0.001) in blood glucose levels from (105 ±5 mg/dL) in control to (450±18 mg/ml) in diabetic rats. In addition there was a meaningful increase (p<0.05) between cratinin, cholesterol, Triglyceride, urea and uric acid levels of diabetic and control mothers’ plasma. Comparing of glomerular plexus volume showed that there was a significant increase (p<0.01) in the glomerular plexus volume from newborns of diabetic mothers in comparison to the control ones. This increase was (1/6 ±0.1) mm3 in control to (2/4±0.1) mm3 in newborns from diabetic mothers. Also Comparing of total length capillaries of glomerular plexus in two groups showed that there was a significant increase (p<0.01) in the total length capillaries from newborns of diabetic mothers in comparison to the Fig 3.
In this work, we aimed to delineate the functional role of maternal diabetes in modulating renal morphogenesis in their offspring. Maternal diabetes presents an environmental challenge in utero and may fundamentally and dynamically impair the process of embryogenesis [15]. Hyperglycemia induces several biochemical and hemodynamic abnormalities [16]. Data indicate that there is a significant change (p<0.05) in the blood glucose, uric acid, urea, Triglycerides and Cholesterol in different groups (Table 1). In newborns from diabetic mothers, there was a significant increase (p<0.05) in the glomeroli plexus volume in comparison to the control ones (Fig. 1). Microvascular systems alteration is responsible for the most devastating complications of diabetic patients. Similar to the microvascular systems of retina, choroid plexus, kidney and glomeroli plexus can be a vulnerable target organ for hyperglycemia. Accumulated data support that oxidative stress induced by chronic hyperglycemia plays a key role in both microvascular and macrovascular complications of diabetes[18]. Our results show that the total length of glomeroli plexus capillary in newborns from diabetic mothers have had a proliferation in number of capillary (P<0.01)(Fig.2). Hyperglycemiamaternal diabetes induce elevated metabolism and hypoxia. These two factors produce Endotelial growth factors such as VEGF and TGF [19]. Indeed it is apparent that a chronic deficiency in kidney new-vessel formation in the face of increasing ischemia makes a major contribution toward progression to the sight threatening proliferate stages of diabetic diseases.

Angiogenesis may be the result of an imbalance between stimulatory and inhibitory factors that presumably accrue from the elevated expression of local angiogenic factor induced by ischemia [20]. Ischemia is common evidence in diabetes, Sharma et al [21] demonstrated the retinal microvascular endothelium shows a threefold increase in cell replication in diabetic rats compared with non diabetic controls. Alteration of vasculature in response to prolonged hyperglycemia may provide specific ligands for vascular targeting in type 1 diabetes [22]. Our finding in this study is correlated with other studies that indicate maternal diabetes can effect on microvascular system such as choroid plexus [11] and glomeroli plexus capillary in newborns. Other data in this study show that there is significant different in the human volume in newborns from diabetic mothers in compare with control mothers (Fig.3). But there is not any significant different between proximal and distal tubules’ diameter in experimental and control groups (Fig.4,5).

According to this data, we could not establish a relationship between the level of hyperglycemia during pregnancy and nephron disstructure in newborns. But induced changes in golomeroli volume and capillaries can lead to renal dysfunction. As studies indicated Uncontrolled hyperglycemia in type 1 diabetic mothers promotes renal malformations in offspring [23]. Evidence suggests that intrarenal RASs within glomeruli and proximal tubules may be activated with hyperglycemia, leading to stimulation of local ANG II production, which may exert feedback inhibition of systemic renin release. Once formed, intrarenal ANG II exerts most of its well-described effects through binding to ANG (1) receptors that are abundantly present in cells of the glomeruli, tubules, vasculature, and interstitium. Thus, ANG (1)-receptor activation increases vascular resistance, reduces renal blood flow, and stimulates production of extracellular matrix in the mesangium and tubulointerstitium [24]. Angiogenices could disturb balance between ANG I and ANG II production therefore be directed to the abnormality in kidney. In total, it is concluded that
maternal diabetes induce angiogenesis in way that glomeruli plexus volume and total length of its capillaries were increased. The present study is high risk for renal dysfunction in newborns from diabetic mothers. Earlier studies have suggested that increased glucose lead to malformation. In addition, oxidative stress disturbances in the polyol pathway and prostaglandin metabolism have been proposed to induce diabetic abnormally. If so, offspring of diabetic mothers may be predisposed to glomerular and vascular diseases

Acknowledgments
Authors would like to thanks the Islamic Azad university Mashhad branch for financial Supports.

References