

Renal echo-3D and microalbuminuria in children of diabetic mothers: a preliminary study

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Maternal diabetes has assumed epidemic relevance in recent years and animal studies have provided some evidence that it may cause abnormalities in renal development and a reduction in nephron endowment in the offspring; however, human data are lacking. The renal cortex contains ~95% of the glomeruli and its volume could be taken as a surrogate measure of glomerular number; based on this assumption, we measured renal cortex volume and in addition, microalbuminuria in a homogeneous sample of 42 children of diabetic (pregestational, $n = 13$, and gestational, $n = 29$) mothers, compared with 21 healthy children born of non-diabetic mothers. The offspring of diabetic mothers showed a significant reduction of renal cortex volume and higher albumin excretion compared with controls, possibly attributable to a reduction in the number of nephrons and the difference was statistically significant ($P < 0.001$). Although further studies on a larger sample are necessary, our preliminary findings suggest that maternal diabetes may affect renal development with sequelae later in life, requiring closer monitoring and follow-up. Furthermore, the importance of strict maternal diabetes management and control must be emphasized.

Received 4 November 2012; Revised 16 February 2013; Accepted 20 February 2013; First published online 10 April 2013

Key words: diabetes, microalbuminuria, renal cortical volume, renal development

Introduction

Human nephrons are being formed starting at the 5th week of gestation and this process is completed by the 34th–36th week so that the number of glomeruli (Nglom) is definitely established for each individual at birth and no more nephrons can be formed for the rest of life.^{1,2} Several factors may affect such a process including maternal malnutrition, utero-placental insufficiency and diseases such as diabetes, leading to a reduced nephron endowment and possibly to an increased risk of hypertension and renal dysfunction in adult life.^{1–5} As far as diabetes is concerned, it has reached epidemic proportions worldwide and dysregulation of glucose metabolism is found in up to 15% of pregnancies according to IADSPG;⁶ despite such impressive numbers only a few studies, mostly performed in laboratory animals,^{7–11} have examined the impact of maternal diabetes on nephron endowment; in murine models maternal diabetes was found to induce a 40% reduction of nephron endowment at birth and to lead to hypertension,

albuminuria, and renal injury, which could be prevented, at least in part, by insulin treatment. Induced maternal diabetes in pregnant rats reduced Nglom in male offspring.^{10,11} A recent study, performed in humans,¹² compared non-diabetic offspring of type 1 diabetic mothers to a control group of offspring of diabetic fathers; the authors were able to show a diminished renal functional reserve in the former group, interpreting this finding as the consequence of a reduction of Nglom.

Counting nephron number and/or measuring glomerular size by non-invasive methods is currently not feasible in humans and thus, as ~95% of glomeruli are located in the renal cortex, renal cortical volume and birth weight have been proposed as surrogate measures of Nglom.^{13–15}

Data from biopsies and from experimental models suggest that a reduction in nephron number, even when compensated by glomerular hypertrophy, may be a major mechanism that induces hypertension and renal failure in adult life;^{2–5} however, clinical data on the latter are scarce.

Microalbuminuria (μA) arises from increased leakage of albumin through the glomerular filtration barrier because of changes in its physico-chemical properties and glomerular endothelial dysfunction, and it is a powerful predictor of cardiovascular disease and mortality in adults and is considered to

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be an established predictor of chronic kidney disease; μA has also been associated with hyperfiltration in children with type 1 diabetes.^{16,17}

Aim of the study

Based on the aforementioned premises we aimed at ascertaining in a preliminary study if maternal diabetes may affect renal cortical volume in the offspring, irrespective of birth weight, and whether this may also coincidentally affect albumin excretion in children of diabetic mothers compared with children of non-diabetic controls, evaluated at 3 years of age.

Subjects and methods

We studied 63 children, 36–39 months old, born at term in our Hospital after an uncomplicated pregnancy: 42 children (overall diabetes, OD) were born of mothers with diabetes mellitus (two with type 1, 11 with type 2, 29 with gestational diabetes (GDM)), and 21 controls born of non-diabetic women without any risk factor for renal diseases (Table 1), who had normal blood glucose levels throughout pregnancy and a normal oral glucose tolerance test, performed between the 24th and 28th week of gestation; three women in the OD group were of North-African and one of Asian descent, whereas one control was of North-African descent, with all the remaining being Caucasians; none of the women had any renal risk factor other than diabetes. Thirty-two women in the diabetes group and 16 among the controls were primiparas; all the patients in the OD group were treated with insulin, but four who were treated with diet alone. Birth weight and growth percentiles at age of examination (Table 1) were comparable in the three groups and none of these children were born at gestational age <35 weeks. In all children, we evaluated renal cortical volume (in ml) by echo-3D, combined with QLAB volume software, a general imaging 3D quantification software (Philips, USA). We estimated the volume as the average of four repeated measures performed by a biomedical engineer blinded to group assignment; all measurements were performed by the same sonographer with intra- and inter-operator variability =4.0 and 5.1%, respectively (coefficient of variation).

We measured μA (mg/dl) as the average of two samples, taken on non-consecutive mornings, by means of the Beckman-nephelometric method.

The process for obtaining informed consent was approved by the appropriate institutional review committee.

Statistical analysis

The Kolmogorov–Smirnov test was used to assess normality of distribution of continuous variables. The Mann–Whitney *U*-test and Kruskal–Wallis test, followed by the Dwass–Steel–Chritchlow–Fligner test for multiple comparisons were used. Proportions were compared by Fisher's exact test.

All calculations were carried out with Predictive Analytic Software (PASW, USA, 2009).

Results

The study findings are summarized in Table 1 and Fig. 1. The children of mothers affected by diabetes (independently of the type 1, 2 or GDM), showed a lower renal cortical volume; no correlation was found among maternal BMI, cortical volume and μA values ($\rho = -0.22$, $P = 0.867$; $\rho = -0.17$, $P = 0.898$, respectively).

No correlation was found among the OD birth weight, cortical volume, and μA values ($\rho = -0.166$, $P = 0.194$; $\rho = 0.055$, $P = 0.668$). Cortical volume did not differ significantly in the female and male children, either in the control (18.1 ml, range 16.1–19.2 *v.* 17.3 ml, range 15.6–21.6, $P = 0.28$) or in the diabetes group (15.9 ml, range 6.2–19.3 *v.* 15.8 ml, range 10.5–24.1, $P = 0.68$); a slight, non-significant, inverse correlation was observed between renal cortical volume and HbA_{1c} levels ($\rho = -0.15$, $P = 0.24$). Finally, no correlation was observed between renal cortical volume and children BMI ($\rho = -0.14$, $P = 0.25$), whereas a borderline correlation ($\rho = 0.21$, $P = 0.09$) was found with maternal BMI.

Discussion

Even though the sample studied was relatively small the groups were comparable as far as birth weight, gestational age and growth percentile are concerned, with values within the normal range at the age of the examination. The children of diabetic mothers showed a significantly lower cortex volume compared with controls; such a finding seems to be attributable solely to maternal diabetes, as birth weight was comparable between groups with no difference between gestational and pregestational diabetes in this respect.

How maternal diabetes may affect renal cortical volume and thus, possibly Nglom, is still a matter of debate: several mechanisms have been proposed, among which down regulation of the IGF-1-mannose-6-phosphate receptor expression, activation of NF- κ B, or the so-called 'podocyte insufficiency'.² In murine models activation of the intrarenal renin-angiotensin system and TGF- β 1 gene expression have been implicated in maternal diabetes-induced renal damage and in the process of fetal programming of adult hypertension.^{10,11} There is growing evidence that early life malnutrition can cause epigenetic damage by inducing persistent changes in DNA methylation,^{18,19} but whether this mechanism applies to maternal diabetes it is not known. Human data on the fetal programming of renal function by maternal diabetes are lacking. To our knowledge, the only available evidence comes from a carefully conducted study¹² of renal function in offspring of type 1 diabetic mothers, which, compared with controls showed the former to have a decreased renal plasma flow and glomerular filtration rate (GFR) response to the infusion of amino acids; the authors interpret such a

Table 1. Clinical, demographic and lab parameters regarding the children and their mothers; children renal cortical volumes measured at age 3

	Type 1–2 diabetes (n = 13)	p ^c	Gestational diabetes (n = 29)	Overall diabetes (n = 42)	p ^d	Control subjects (n = 21)	p ^e	p ^f
Maternal age (years) ^a	35.0 ± 3.4	0.989	34.5 (7.1–9.7)	34.9 ± 3.9	0.001	30.2 ± 5.0	0.001	0.039
Maternal BMI (kg/m ²) ^b	20.9 (18.8–31.8)	0.066	26.5 (18.4–45.1)	24.2 (18.4–45.1)	0.304	23.0 (20.0–28.0)	0.091	0.437
Current smokers (%)	0		10.3	7.1		9.5		
Mean HbA _{1c} (%) ^b	7.2 (5.6–9.1)	0.001	6.0 (5.3–7.0)	6.2 (5.5–9.1)		NA		
Body weight gain (kg) ^a	11.5 ± 6.4	0.44	10.1 ± 6.8	10.9 ± 6.2	0.22	13.6 ± 3.9	0.27	0.37
Neonate birth weight (g) ^b	3500 (2670–3990)	0.962	3450 (2575–4025)	3462 (2575–4025)	0.998	3350 (2630–4040)	0.997	0.966
Gestational age (weeks) ^b	38 (36–41)	0.981	38 (36–40)	38 (36–41)	<0.001	39 (37–41)	<0.001	0.005
Weight percentile	55 (40–90)	0.89	59 (20–95)	55 (20–95)	0.90	55 (35–70)	0.88	0.90
Height percentile	50 (40–65)	0.86	55 (20–75)	53 (20–75)	0.85	55 (20–70)	0.88	0.91
Renal cortex volume of right kidney (ml) ^b	15.7 (6.5–17.4)	0.504	15.8 (6.4–25.6)	15.7 (6.4–25.6)	<0.001	17.3 (16.0–22.1)	0.008	<0.001
Renal cortex volume of left kidney (ml) ^b	15.7 (6.5–18.8)	0.572	16.0 (6.0–22.6)	15.8 (6.0–22.6)	<0.001	17.9 (14.7–21.1)	0.004	0.001
Renal cortex volume mean of both kidneys (ml) ^b	15.8 (6.5–17.4)	0.581	15.9 (6.2–24.1)	15.8 (6.2–24.1)	<0.001	17.9 (15.6–21.6)	0.005	<0.001
Urinary albumin excretion (mg/dl) ^b	0.540 (0.200–1.000)	0.052	0.320 (0.200–2.800)	0.385 (0.200–2.800)	<0.001	0.200 (0.200–0.300)	<0.001	<0.001

BMI, body mass index.

^a Mean ± S.D.

^b Median and range.

^c Type 1-2 diabetes *v.* gestational diabetes.

^d Tverall diabetic *v.* controls.

^e Controls *v.* type 1–2 diabetes.

^f Controls *v.* gestational diabetes.

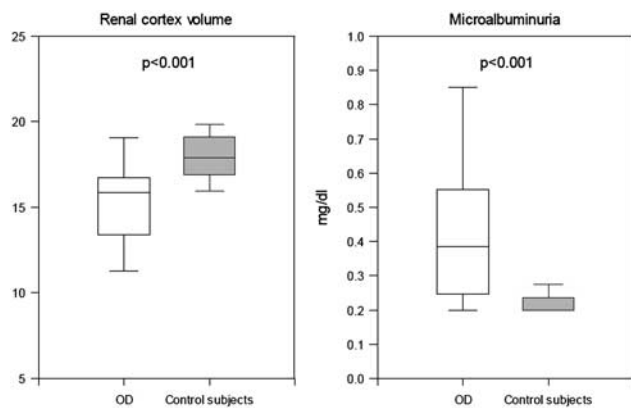


Fig. 1. Box and whisker plots of renal cortical volume and microalbuminuria in children of diabetic mothers (OD), and controls.

finding as a reduced functional reserve that in turn might reflect a reduced number of nephrons, undergoing individual hyperfiltration.

We found higher μA levels in the children of diabetic mothers; we are tempted to speculate that it might be secondary to an increased single nephron GFR, although, and this is a limitation of our study, we did not measure GFR; however, a recent study,²⁰ performed in low-birth-weight infants showed despite a smaller kidney volume, a GFR similar to controls, indicating that hyperfiltration begins in early life.

We were not able to show any gender-related difference in renal cortical volume. This could be due to the small sample and/or to the fact that some of the girls in the OD groups were overweight^{2,17} and were born of obese mothers (BMI > 35) who had not reached the target of treatment of diabetes.

Conclusions

The current therapeutical approaches have reduced the incidence of perinatal risks in offspring, but the challenge for the future is to reduce the long-term incidence of diabetes, obesity, hypertension and chronic kidney disease as well.

Our data suggest that maternal diabetes may have adverse effects on the kidney in the offspring. The findings of our study may contribute a further rationale to pursue an early diagnosis and/or strict control of diabetes in pregnancy, as children of diabetic mothers constitute an ever increasing population that may be overly susceptible to renal disease in adult life.

Acknowledgments

None.

Financial support

None.

Conflict of interest

None of the authors report a conflict of interest. They all disclosed no conflict of interest of a financial or other nature, and they all agreed with this final version. The results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another journal.

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