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SHORT COMMUNICATION

Serum creatinine during physiological perinatal dehydration may estimate individual nephron endowment

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Abstract

It is well known that the nephron endowment of healthy subjects is highly variable and that individual nephron mass has potentially important implications both in health and disease. However, nephron count is technically impossible in living subjects. Based on the observation of an increase in serum creatinine (sCr) in otherwise healthy newborns with solitary kidney during the physiological perinatal dehydration, we hypothesized that perinatal sCr might be helpful in identifying healthy subjects with a reduced nephron mass. In the framework of a study on blood pressure in babies (NeoNeph), sCr of normal Caucasian neonates was determined 48–96 h after birth and their association with a family history of arterial hypertension (AH) was analyzed. SCr was determined in 182 normal newborns (90 males) at a mean of 61 ± 8 h after birth (range 46–82). Newborns with paternal AH had a higher mean sCr (0.97 + 0.28 mg/dL) then newborns without paternal AH (0.73 + 0.28 mg/dL; $p = 0.006$). No differences in mean sCr were found in relation with mother or grandparent's history of AH.

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Conclusion: The association between parental AH and high sCr during perinatal dehydration supports the hypothesis that the latter is a promising tool for identifying normal subjects with a reduced nephron mass with potential important implications in prevention and in understanding the individual outcome of renal and extrarenal diseases (including AH).

What is Known:

• *Nephron endowment of healthy subjects is highly variable and individual nephron mass has potentially important implications both in health and disease however nephron count is not feasible in living subjects.*

What is New:

• *Serum creatinine during perinatal dehydration is a possible biomarker for identifying normal subjects with a reduced nephron mass.*

Keywords Nephron endowment · Newborn · Arterial hypertension · Blood Pressure · Perinatal serum creatinine

Introduction

The concept of individual nephron endowment has potentially important implications for both health and disease. The outcome of renal diseases, the toxicity arising from drug exposure, and the risk of arterial hypertension may all be very different depending on the number of nephrons at birth given that the entire complement of the human kidney is determined by 36 weeks' gestation, and nephrons do not regenerate [6, 11, 20].

It is well known that the endowment of healthy subjects varies widely from a minimum of a few hundred thousand nephrons per kidney to a maximum of over two millions, although it is not technically possible to count them except in the setting of a post-mortem examination [7, 16–19, 22]. During physiological perinatal dehydration, the serum creatinine (sCr) levels of subjects born with monolateral renal agenesis (who have fewer nephrons by definition) are much higher than those observed in neonates with two kidneys, but subsequently (even as early as the first month of life), they become indistinguishable from those of the general population [12, 21]. On the basis of this simple observation, we hypothesized that serum creatinine levels 48–72 h after birth (when healthy babies usually reach the nadir of postnatal body weight) might help to identify subjects with a reduced nephron mass. On the other hand, a reduced number of nephron and the consequent reduced capacity to excrete the common excessive salt intake have been often related to salt-sensitive hypertension which typically affects younger people [1]. We therefore measured serum creatinine 2–4 days after delivery in a series of newborns and evaluated its association with the family history of arterial hypertension (AH).

Methods

After obtaining the informed consent of their parents/guardians, healthy and appropriate for gestational age Caucasian neonates born at term after an uneventful pregnacy (37–41 weeks) underwent sCr determinations at the time of the expanded newborn screening test. Birth weight, weight loss, and family history of AH (parents and grandparents) were recorded by means of an "ad hoc" questionnaire. Exclusion criteria were any mother's disease or any pathological event during pregnancy including need of medications, any proven or suspected disease in the baby (all patients had performed ECHO scan screening at 20th–22nd gestational week (GW)) including birth weight < 2500 or > 4400 g or premature birth (< 37th GW) and supra-physiological perinatal dehydration (> 13% of birth weight).

Blood samples were taken within 2–4 days after delivery. The study was approved by the Ethics Committee of our Institution. The determination of sCr, which was repeated at 1 year of age, was performed by means of a LC-MS/MS validated method from the dried blood spots normally used for newborn screening [3, 5].

The Mann-Whitney test and multiple linear regression models (with a robust standard error because of the slightly non-normal distribution of sCr levels) were used to correlate sCr levels of newborns with the family history of AH. The adjustment covariates were gender of the newborn, absolute weight loss (grams), and weight loss velocity (grams/h) between birth and the time of blood sampling.

Analyses were performed using Stata 13 (StataCorp. 2013. Stata: Release 13. Statistical Software. College Station, TX: StataCorp LP).

Results

SCr levels were determined a mean 61 ± 8 h after birth (range 46–82) in 182 normal (90 males) Caucasian newborns whose mean birth weight was 3301 ± 374 g (range 2495–4345). By the time sCr was measured, their mean weight loss had been 253 ± 78 g (range 25–510), equivalent to a total mean weight loss of 7.7 ± 2.3% or a loss per hour of 0.13 ± 0.04% of their birth weight (range 0.01–0.24).

The median sCr and interquartile range (IQR)), as determined during perinatal dehydration, was 0.72 mg/dL (0.56–0.95) without any significant difference by gender; its relation with family history of AH is shown in detail in Table 1. The 11 newborns with paternal AH, out of which 8 (73%) were males, had a higher

Table 1 Mean (SD) serum creatinine levels during perinatal dehydration in 182 consecutive newborns according to arterial hypertension family history

Family history of AH	N*	Mean (SD) sCr (mg/dL)	Crude *p* value**	Adjusted *p* value***
Mother				
Without AH	179	0.75 (0.29)	0.61	0.19
With AH	2	0.78 (0.01)		
Father				
Without AH	169	0.73 (0·28)	0.005	0.006
With AH	11	0.97 (0·28)		
Maternal grandmother				
Without AH	123	0.76 (0.28)	0.76	0.71
With AH	57	0.74 (0.31)		
Maternal grandfather				
Without AH	105	0.75 (0.27)	0.46	0.58
With AH	63	0.72 (0.30)		
Paternal grandmother				
Without AH	106	0.72 (0.26)	0.50	0.51
With AH	49	0.76 (0.29)		
Paternal grandfather				
Without AH	105	0.76 (0.31)	0.25	0.12
With AH	63	0.70 (0.28)		

AH arterial hypertension, *sCr* serum creatinine

*Newborns with missing information on AH in the family not included

**From Mann-Whitney tests

***From multiple regression models with robust standard error, including the covariates gender of the newborn, absolute weight loss (grams), and weight loss speed (grams/h) between birth and the time of blood sampling

Note: Conversion factors for units: serum creatinine in mg/dL to mol/L, × 88 .4

mean sCr level then newborns without paternal AH in either crude ($p = 0.005$) or adjusted analysis ($p = 0.006$) (Fig. 1). No differences in mean sCr levels were found in relation with mother's or grandparents' history of AH, nor with the number of affected parents/grandparents. The sCr level was redetermined at 1 year of age and all values were within the normal range; in detail, the median value was 0.34 (IQR 0.29–0.47) without any correlation between the level recorded perinatally and 1 year later. During the very first days of life, only 6% of the newborns were exposed to formula feeding and the relative distribution was not different in those born to normotensive or hypertensive fathers.

Discussion

In Western countries, approximately 7% of the general population have moderate chronic kidney disease (CKD), and this high frequency cannot be explained by immunological or genetic diseases because they only affect less than 0.01% of the population. The most common cause of CKD is nephroangiosclerosis, which is also responsible for one-third of the cases of end-stage renal disease (ESRD). This condition is due to the involvement of the kidney vasculature in the atherogenic disease which, as correctly stated by Zoccali, drives the high rate of cardiovascular morbidity in industrialized countries [25]. The potential role of a reduced number of nephrons in the increased risk of hypertension and its cardiovascular consequences, as well as in progressive kidney diseases, has been clearly documented: rat strains with a large number of nephrons are less susceptible to the development of CKD, and conversely, a reduced number of nephrons are associated with hypertension and CKD [8, 10, 13, 14]. Back in 1988, Brenner et al. postulated that kidneys with fewer nephrons and a consequently small filtration surface area have less sodium excretion capacity, thus inducing hypervolemia as a consequence of high dietary sodium intake which can turn into the development of hypertension [4]. In this theoretical setting, the small number of nephrons is a condition that predisposes to hypertension which, once developed, contributes to damage other nephrons (nephroangiosclerosis), thus creating a vicious circle that leads to a further reduction in the already limited number of nephrons and the development of CKD and finally ESRD. Nowadays, it is known that a number of metabolites that accumulate in the case of a reduced nephron mass (such as phosphate, uric acid, parathyroid hormone, fibroblast growth factor 23, and others) may be responsible for vascular damage and thus further contribute to the spiral leading to ESRD and cardiovascular morbidity [23, 24].

Moreover, it can be presumed that subjects with fewer nephrons are more susceptible to the renal or systemic effects of exogenous toxic agents (nephrotoxic drugs) and endogenous (immunological) assault. The possibility of correlating a significant part of the different outcomes of these conditions with differences in nephron endowment has not been extensively explored because of the lack of reliable means of categorizing subjects a priori, on the basis of their number of nephrons.

The rationale of the present study was based on the frequent observation, by neonatal nephrologists, that otherwise healthy subjects with unilateral renal agenesis, whose sCr levels under normal conditions are well within the normal range, are much more likely to show significantly higher levels as a result of minor dehydration than subjects with two kidneys. The same is true of patients with congenital abnormalities of the kidney and urinary tract (CAKUT), who have fewer nephrons because of renal hypodysplasia [12, 21].

In addition to being intuitively consistent with the general knowledge of renal physiology, our hypothesis that sCr levels during physiological dehydration may reflect individual nephron endowment is indirectly supported by the finding of a significantly higher sCr level in neonates born to hypertensive parents, given the well-documented concept that hypertension

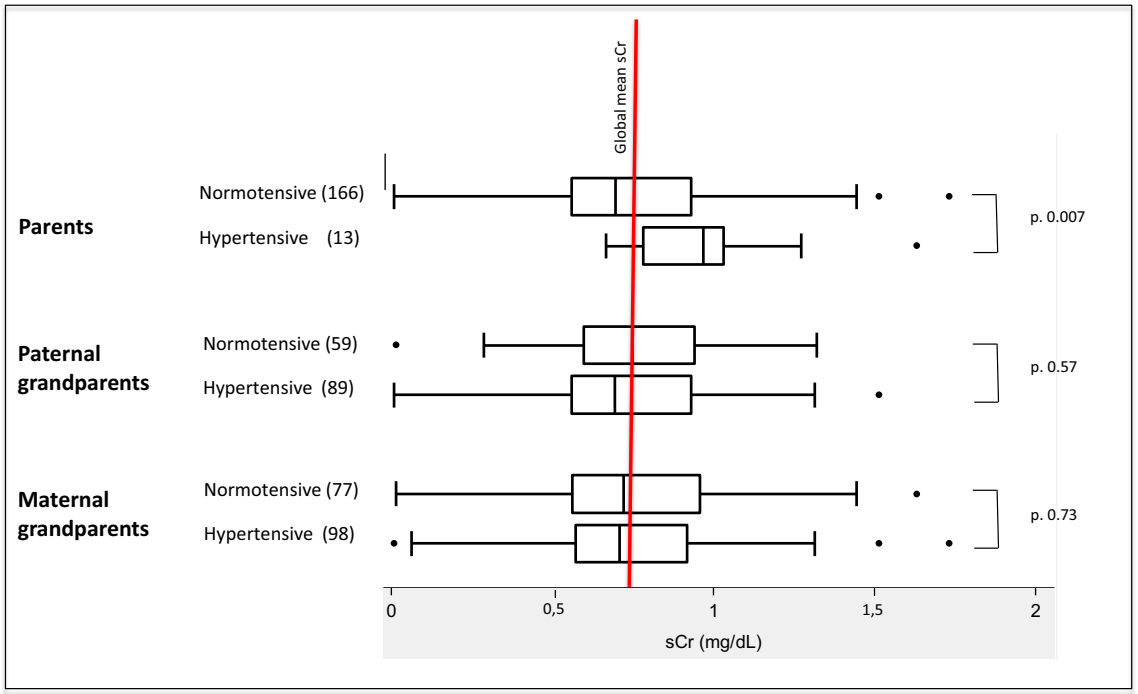

Fig. 1 Perinatal serum creatinine during perinatal dehydration according to family history of arterial hypertension

in younger subjects is associated with a reduced number of nephrons in otherwise healthy young subjects [14, 15].

It is interesting that the association between blood pressure in relatives and sCr during dehydration is observed in children with hypertensive fathers only, not with hypertensive mothers or grandparents. As far as mothers are concerned, the number of subjects with AH was very small (thus insufficient for statistical significance), perhaps because they were quite younger than fathers (34.7 ± 4.5 vs 37.2 ± 5.6 years; $p < 0.001$). Finally, it is known that arterial hypertension of the elderly has a different ethiological pattern (atherosclerosis) than that of young adults in whom low nephron mass might be more relevant [2, 9].

We are aware that, being based on relatively small numbers and on indirect evidences, our findings are preliminary but, to the best of our knowledge, our hypothesis has never been postulated before, and the possibility of identifying subjects with a reduced number of nephrons very early in life and by means of a simple test not only opens up a series of preventive opportunities, but also allows for new interpretations and speculations concerning important causes of morbidity and their variable outcomes in the adult population. The findings may also provide other researchers a clue for a better understanding of disease mechanisms and ways of promoting health. If they are confirmed and extended with follow-up data tracking arterial blood pressure in this and other cohorts of children, it might be worth considering a neonatal screening program focused on the risk of CKD. All the metabolic diseases for which a screening program is a standard of care in many countries are much less common than hypertension, CKD and the consequent cardiovascular morbidity.

Another potential critical issue in the present study is the use of sCr for estimating renal function which is perhaps not the best biomarker in newborns for a number of possible interferences (bilirubin, muscular mass, mother's sCr, etc). This choice, although questionable, was determined by the impossibility of proposing venopuncture in a study setting on healthy newborns which would not have been accepted by parents. SCr determination on blood drop taken at time of the metabolic screening was the only acceptable compromise. Nevertheless, our main finding is based on comparison rather than on absolute values, minimizing the potential bias (including those derived from maternal sCr).

In conclusion, the association among parental AH and high sCr during perinatal dehydration supports the hypothesis that the latter is an interesting possible tool for identifying subjects with a reduced nephron mass with potential important implications in understanding the individual outcome of renal diseases (including drug toxicity), expanding our knowledge on the pathophysiology of common renal and extrarenal diseases, and providing clues for individualized preventive measures.

The physiological dehydration of newborns is a unique event in which pure dehydration takes place without any concomitant confounding metabolic conditions that might interfere with the interpretation of renal function adaptation. Furthermore, the neonatal period is also unique in terms of parental receptiveness to health promotion messages provided that these are not generic but individualized to the specific needs of their own children.

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Authors' contributions Gianluigi Ardissino, MD, PhD, conceptualized and designed the study, analyzed the data, drafted the initial manuscript, and revised and approved the final version.

Francesca Tel designed the data collection instruments, performed data collection, revised the article, and approved the final version.

Michela Perrone designed the data collection instruments, performed data collection, and the editing of the manuscript.

Ilaria Possenti performed data collection, edited, revised, and approved the final version.

Mariangela Pavesi performed kidney ultrasonography and revised and approved the final version.

Giulia Forni performed laboratory assessment and revised and approved the final version.

Patrizia Salice performed data collection and revised and approved the final version.

Lorenzo Colombo performed data collection and revised and approved the final version.

Stefano Ghirardello performed data collection and revised and approved the final version.

Bianca Castiglione performed data collection.

Dario Consonni performed the statistical analysis and revised and approved the final version.

Laura Baca performed data collection and revised and approved the final version.

Daniela Li Vecchi performed data collection and revised and approved the final version.

Giancarlo la Marca performed laboratory assessment and revised and approved the final version.

Fabio Mosca contributed in conceptualizing and designing the study and revised and approved the final version.

Compliance with ethical standards The study was approved by the Ethics Committee of our Institution.

Conflict of interest The authors declare that they have no conflict of interest.

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