Relationship between renal volume, prematurity, birth weight and retinal microvasculature

Dr. Yogavijayan Kandasamy MBBS, M. Clinical Epidemiology

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Mothers and Babies Research Centre Priority Research Centre for Reproductive Science School of Medicine and Public Health Faculty of Health University of Newcastle

Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Acknowledgement of Authorship

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

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Yogavijayan Kandasamy

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"Try not. Do or do not. There is no try"

Jedi Master Yoda

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Supervisors

Professor Dr Roger Smith Director, Mothers and Babies Research Centre Priority Research Centre for Reproductive Science School of Medicine and Public Health Faculty of Health University of Newcastle

Associate Professor Dr Ian M. R. Wright Senior Neonatologist/Associate Professor in Paediatrics and Child Health Mothers and Babies Research Centre Priority Research Centre for Reproductive Science School of Medicine and Public Health Faculty of Health University of Newcastle

List of Publications Included as Part of the Thesis

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- Kandasamy Y, Smith R, Wright IMR. Retinal microvascular changes in low-birthweight babies have a link to future health. *Journal of Perinatal Medicine*. 2012; 40(3):209-14.
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- Kandasamy Y, Smith R, Wright IMR. Measuring Cystatin C to Determine Renal Function in Neonates. *Pediatric Critical Care Medicine* 2013;14(3):318-22
- Kandasamy Y, Smith R, Wright IMR, Hartley L. Pain relief for premature babies during ophthalmology assessment. *Journal of the American Association for Pediatric Ophthalmology and Strabismus (JAAPOS)*. 2011; 15(3):276-80.

Original research articles

- Kandasamy Y, Smith R, Wright IMR. Retinal microvasculature measurements in fullterm newborn infants. *Microvascular Research*. 2011; 82(3):381-4.
- Kandasamy Y, Smith R, Wright IMR, Hartley L. Relationship between birth weight and retinal microvasculature in newborn infants. *Journal of Perinatology*. 2012; 32(6):443-7.
- Kandasamy Y, Smith R, Wright IMR. Relationship between the retinal microvasculature and renal volume in low-birth-weight babies. *American Journal* of Perinatology. 2012; Sept 21.
- Kandasamy Y, Smith R, Wright IMR, Lumbers ER. Relationships between glomerular filtration rate (GFR) and kidney volume in low-birth-weight neonates. *Journal of Nephrology*. 2012; Oct 3.
- Kandasamy Y, Smith R, Wright IMR, Lumbers ER. Extra-uterine renal growth in preterm infants. (undergoing peer review)

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Statement of Contribution of Others

Co-author statement

I, Prof. Roger Smith, attest that Research Higher Degree candidate Yogavijayan Kandasamy contributed substantially in terms of study concept and design, data collection and analysis, and preparation of these manuscripts:

- Kandasamy Y, Smith R, Wright IMR. Retinal microvascular changes in low-birthweight babies have a link to future health. *Journal of Perinatal Medicine*. 2012; 40(3):209-14.
- Kandasamy Y, Smith R, Wright IMR. Oligonephropathy of Prematurity. American Journal of Perinatology. 2012; 29(2):115-20.
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(Signature of Co-Author) (Prof. Roger Smith) Date: 25/10/2012

(Signature of Candidate) (Yogavijayan Kandasamy) Date: 25/10/2012

I, Assoc. Prof. Ian MR Wright, attest that Research Higher Degree candidate Yogavijayan Kandasamy contributed substantially in terms of study concept and design, data collection and analysis, and preparation of these manuscripts:

- Kandasamy Y, Smith R, Wright IMR. Retinal microvascular changes in low-birthweight babies have a link to future health. *Journal of Perinatal Medicine*. 2012; 40(3):209-14.
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(Signature of Co-Author) (Assoc. Prof. Ian MR Wright) Date: 25/10/2012 (Signature of Candidate) (Yogavijayan Kandasamy) Date: 25/10/2012

I, Emeritus Prof. Eugenie R. Lumbers, attest that Research Higher Degree candidate Yogavijayan Kandasamy contributed substantially in terms of study concept and design, data collection and analysis, and preparation of these manuscripts:

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(Signature of Co-Author) (Emeritus Prof. Eugenie R. Lumbers) Date: 25/10/2012

(Signature of Candidate) (Yogavijayan Kandasamy) Date: 25/10/2012

I, Dr. Leo Hartley, attest that Research Higher Degree candidate Yogavijayan Kandasamy contributed substantially in terms of study concept and design, data collection and analysis, and preparation of these manuscripts:

- Kandasamy Y, Smith R, Wright IMR, Hartley L. Pain relief for premature babies during ophthalmology assessment. *Journal of the American Association for Pediatric Ophthalmology and Strabismus (JAAPOS)*. 2011; 15(3):276-80.
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(Signature of Co-Author) (Dr Leo Hartley) Date: 25/10/2012

(Signature of Candidate) (Yogavijayan Kandasamy) Date: 25/10/2012

Table of Contents

Statement of Originality	ii
Acknowledgement of Authorship	ii
Acknowledgements	iii
Supervisors	v
List of Publications Included as Part of the Thesis	vi
Review articles	vi
Original research articles	vi
Copyright statement	vii
Statement of Contribution of Others	viii
Co-author statement	viii
Table of Contents	xiv
List of Figures	xv
Abstract	xvi
Chapter One – Overview	1
Introduction	1
Intrauterine growth restriction and small for gestational age	2
Low birth weight	2
Consequences of premature birth and low birth weight	4
Chapter Two – Research Methodology	6
Hypothesis	6
Aims	6
Objectives	6
Method: Study design	6
Sampling technique	7
Subgroup analysis	7
Renal ultrasound	8
Renal function	9
Retinal examination	9
Method	10
Retinal image analysis	11
Outcome measurements	11
Patients recruited for this study	11
Chapter Three – Published Papers	14
Review articles	14
Original research articles	75
Chapter Four – Conclusions and Future Direction	144
Appendices	146
Appendix A: Permission to use a quote	146
Appendix B: Licences to reproduce images and figures	147
Bibliography	151

List of Figures

Abstract

This thesis consists of a collection of articles arising from a research project carried out as a requirement of a higher research degree at the University of Newcastle. This research project was carried out in a tertiary perinatal centre in North Queensland, Australia. The study cohort consists of three groups – normal and low-birth-weight term infants, and premature infants. In this study, the relationship between renal volume, birth weight and retinal microvasculature was investigated in a cohort of low-birthweight term babies. This study also investigated the relationship between prematurity and extra-uterine renal growth and renal function. For the purpose of assessment of renal function Cystatin C was used, which is a relatively new marker of renal function in infants. Retinal imaging technique and technology used in this study are similar to those used in detecting Retinopathy of prematurity. However, the analysis of retinal microvasculature was limited to term infants. The literature review for this manuscript is presented as a series of published review articles. The results are presented in the format of original research manuscripts, which have been published in peer-reviewed journals. From this study we were able to demonstrate that low birth weight and prematurity result in different yet important changes to the developing kidney. We also demonstrated that while eyes, kidneys and blood vessels are distinct organs, they share common features in low-birth-weight term infants.

Chapter One – Overview

Introduction

The World Health Organization (WHO) defines prematurity as birth before 37 completed weeks of gestation (1). Premature infants can be further subdivided into late preterm (32-36 completed weeks of gestation), very preterm (28 and less than 32 weeks) and extremely preterm (less than 28 weeks) (2). Prematurity is recognised as a condition in which the fetus is biologically immature for extra-uterine life (3). A fetus develops and matures *in utero* until the time at which its organs are ready to sustain extra-uterine life independently. Full-term newborn infants have basic needs that care-givers must fulfil, such as warmth and nutrition, but they are capable of spontaneous respiration, they have the ability to suck and swallow liquid nutrition, and they can indicate when they need nutrition. Essential physiological capabilities, such as metabolism, fluid and electrolyte balance, blood pressure control, and the excretory mechanism function spontaneously (3). Preterm infants are born with immature organs; hence, they need nutritional and respiratory support to survive. In 1960, a neonate weighing 1000 g had a 95% chance of death. Today, an infant of similar weight has a 95% chance of survival (4).

As there are not good direct measures of the degree of maturity, gestational age is used as a proxy measure for the degree of maturity (3). Gestational age can be determined by maternal last menstrual period, obstetric examination and ultrasonography of the fetus (3). It can be unreliable to use the last menstrual period (LMP) by itself to determine fetal gestational age (5, 6). Although menstrual history can be obtained in almost 90% of pregnancies, up to half of these dates will be unreliable (5). The prenatal ultrasound is the most commonly used method for determining the gestational age of a fetus (7, 8). Fetal growth charts have been developed and used to estimate the gestational age based on measurements of various fetal parts, such as the femur length and the head and abdominal circumference (3, 7). An ultrasound performed in the first trimester is the most accurate predictor of gestational age (5, 8). Ultrasound is very accurate (within 7 to 10 days) for determining fetal gestational age if performed in the first trimester (8). However, ultrasound scans become inaccurate in the later part of pregnancy, with up to a 2 to 3 week margin of error in the third trimester (7). Gestational age and maturity of a newborn can also be determined after birth using both the physical appearance and neurological maturation of the newborn.

Worldwide, the number of premature births is estimated to be approximately 14 million per year, and this number is increasing (9). The preterm birth rate is between 5% and 7% in some developed countries, but the rate is reported to be even higher in developing countries (9). Premature birth may occur from preterm labour with intact fetal membrane, the preterm rupture of fetal membrane, or from iatrogenic preterm birth for fetal or maternal indications (2). The pathophysiology of spontaneous premature birth has yet to be fully understood (2), but it is recognised that while there could be multiple underlying precipitating factors, these reasons have a common biological pathway that lead to preterm labour, preterm rupture of membrane, cervical insufficiency and birth.

Intrauterine growth restriction and small for gestational age

Intrauterine growth restriction (IUGR) is defined as the failure of the fetus to achieve its genetic growth potential *in utero* (8). Small for Gestational Age (SGA) is defined as a fetus or infant with growth parameters below the 10th percentile for the gestational age (8). There is considerable overlap between SGA and IUGR: an IUGR baby is usually SGA, but not all SGA infants are IUGR. Some infants are SGA because of constitutional factors and have normal placental function (8). There are various population-based growth charts that can be used to determine the growth of a fetus or an infant (10, 11). Infants born with a birth weight between the 10th and 90th percentile are classified as Appropriate for Gestational Age (AGA); infants are classified as Large for Gestational Age (LGA) if their birth weight lies above the 90th percentile for gestational age.

Low birth weight

Infants born with a birth weight of 2500 g or less are classified as low-birth-weight (LBW) infants (1). LBW infants are a heterogeneous group, comprising infants who are premature, with less than 37 completed weeks of gestation, or growth-restricted, with weight below the 10th percentile for their gestational age, or a combination of both. Infants can be further classified based on birth weight into the following two categories:

very low-birth-weight (VLBW) infants, whose birth weights are less than 1500 g, and extremely low-birth-weight (ELBW) infants, whose birth weights are less than 1000 g (3). Figure 1 provides a summary of how an infant may be classified based on birth and/or gestational age.

Fetal weight estimation has a \pm 15% margin of error, even with the best ultrasound machine and technique (12). On average, 71% of Australian women undergo their first antenatal check during the first trimester (13). In certain parts of regional Australia such as North Queensland, Australian Aboriginal and Torres Straits Islanders babies make up almost a quarter of the hospital births. However, only about a third of the mothers of these babies present for their first antenatal visit during the first trimester (13). In the absence of accurate gestational age measurement, especially in the absence of a first trimester scan and because of the ease and accuracy of measuring birth weight, many researchers continue to use birth weight cut-offs (<2500 g) to designate infants at risk (3, 11, 13).



Figure 1. Comparison of preterm (<37 weeks of gestation), low-birth-weight (<2500g), and small for gestational age (SGA), infants (Pctl=percentile)(3) (reproduced with permission)



Consequences of premature birth and low birth weight

Figure 2. Estimated distribution of the direct causes of 4 million neonatal deaths for the year 2000 (14) (reproduced with permission)

Apart from being a leading cause of neonatal mortality (Figure 2) (14), premature birth is well recognised as causing both short- and long-term morbidity (2, 15). Infants of the lowest gestation ages are at greatest risk for morbidities. The common problems encountered by premature infants in the early neonatal period include respiratory distress syndrome, severe intraventricular haemorrhage, necrotizing enterocolitis, and late-onset sepsis (16) and bronchopulmonary dysplasia. The commonly encountered long-term problems are neurodevelopmental, including learning difficulties, cognition or developmental delay, cerebral palsy, visual impairment and hearing impairment, and patients often have more than one of these problems (17). There is evidence showing that premature infants have an increased risk of developing insulin resistance, glucose intolerance and higher blood pressure (4, 18). LBW is associated with an increased risk of cardiovascular disease and hypertension (19, 20). Prematurity (21-23) and/or LBW (24-26) are increasingly being recognised to be a risk factor for renal disease in adults. It is well recognized that prematurity causes retinal vascular changes known as retinopathy of prematurity (16). Interestingly, retinal microvascular changes have also been identified in children and adults who were born with LBW (27-29).

The first review article discusses retinal microvascular changes in LBW individuals, the techniques that have been used to examine the retinal microvasculature and the clinical significance of these findings on future health (30). The second review

article discusses the association between prematurity, kidney injury and chronic kidney disease (31). In the third review article, the value of a new kidney function marker (Cystatin C) is discussed (32). The fourth review article discusses pain relief during ophthalmological examination (33). Its relevance to this study is further elaborated in the methodology section.

Chapter Two – Research Methodology

Hypothesis

Kidney volume and function:

- 1. Has correlation with birth weight and gestation
- 2. Has correlation with retinal microvasculature in neonates

Aims

- 1. To determine if the kidney volume and function in premature infants is different to term infants
- 2. To determine if kidney volume and function are influenced by birth weight
- 3. To determine retinal microvascular measurements in term infants
- 4. To determine if retinal microvascular measurements correlate with kidney volume and function in newborn infants

Objectives

- To recruit premature babies ≤32 weeks of gestation admitted to the Neonatal Intensive Care Unit
- To carry out assessments of kidney volume, function and retinal microvascular measurements
- 3. To recruit a group of term babies as the control group and carry out similar assessments

Method: Study design

Townsville Hospital is the only tertiary perinatal centre in North Queensland, Australia. The region records 10,000 baby deliveries per year. Premature, LBW and babies needing monitoring are admitted to the neonatal unit. This 12-month study was carried out in this hospital's neonatal unit. The patients were recruited prospectively from August 2010 and the analysis was carried out at the end of the recruitment period. Ethics approval for this study was obtained from the Townsville Health District Human Research and Ethics Committee.

Sampling technique

All babies admitted to the unit during the study period and who fulfilled the inclusion criteria were recruited into the study.

Recruitment of preterm babies

All preterm babies \leq 32 weeks of gestation admitted to the neonatal unit during the study period were eligible to participate in this study. Parents were approached and were given written information by the investigator. Patients were recruited into this study once written consent was obtained.

Inclusion criteria:

All babies \leq 32 weeks of gestation

Parental consent available

Exclusion criteria:

Congenital renal or ocular abnormalities

Chromosomal abnormalities (infants with syndromes)

Recruitment of term babies

Term babies (37 completed weeks to 42 weeks of gestation) admitted to the neonatal unit during the study period were eligible to participate in this study.

Inclusion criteria:

Term babies Parental consent available

Exclusion criteria:

Congenital renal or ocular abnormalities

Chromosomal abnormalities (infants with syndromes)

Infants with respiratory distress who needed respiratory support

Infants of diabetic mothers

Subgroup analysis

Term babies were further divided into groups based on birth weight and gestation:

a. Birth weight:

i. less than 2500 g as low birth weight

ii. 2500 to 4500g as normal birth weight

b. Appropriate for gestational age (birth weight between 10th and 90th percentile)

Sample size calculation for premature infants

Data from a previously published study show the average total kidney volume in the term infant to be 9.6 \pm 2.6 ml (34). Studies in adults born prematurely showed that their kidney volume was 15% smaller when compared with those who were born term (35). For a study with $\alpha = 0.05$ (2-sided), $\beta = 80\%$, 26 patients in each cohort needed to be recruited.

Assessment for preterm babies

At 32 weeks corrected age – renal volume, renal function

At 38 weeks corrected age - renal volume, renal function, retinal examination

Assessment for term babies

Renal ultrasound, renal function measurement and retinal examination

Renal ultrasound

All renal ultrasounds were obtained using the Philips IU22 Ultrasound System (Philips Healthcare, Andover, MA, USA) with a compact (small footprint) curved linear 5–8 MHz frequency transducer. The depth, grey-scale gain, focuses and time-gain compensation were adjusted to acquire the best images of the kidneys. Only one focus was used and this was placed at the level of the kidney. To avoid inter-observer errors during scanning and measuring, the same neonatal sonographer performed all examinations. All infants were examined in the prone position. A sagittal view of the kidney was obtained and the maximum length of each kidney was measured between the uppermost edge of the upper pole and lowest edge of the lower pole. Transverse images of the kidney, the maximum anteroposterior (AP) diameter and transverse diameter (W) were measured (36). Renal length (L), anteroposterior diameter (AP), transverse diameter (W) were measured on both kidneys. Kidney volume (KV)(ml) was calculated according to the following formula: $KV = (\pi/6 \times L \times W \times AP)$ (36). The mean volumes of the right and left kidneys [(Right KV + Left KV)/2] and total KV

(Right KV + Left KV) were also calculated. Coefficient of variation for this measurement is between 24 and 27 % (34).

Renal function

Peripheral venous blood samples (0.5 mL) were drawn from each infant to measure serum creatinine and Cystatin C. Creatinine was measured quantitatively with Beckman Coulter Synchron Clinical Systems using a modified Jaffé method (Beckman Coulter Inc, Brea, CA, USA). Cystatin C was measured by immunoassay using a commercially available technique (Beckman Coulter, Gentian AS, Moss, Norway) (37). Queensland Pathology Laboratory, a facility accredited by National Association of Testing Authorities, Australia (NATA), conducted all analyses.

Retinal examination

A neonatal retinal camera (RetCam) was used to carry out retinal examination.



Figure 3. Neonatal retinal camera (Ret cam II Wide Field Imaging System (Clarity Medical System, Pleasanton, CA 94588, USA)

Method

The retinal imaging technique and technology used in this study is similar to those used in detecting Retinopathy of prematurity (ROP). However, retinal microvasculature analysis in this study was only carried out on term infants. All premature babies < 32 weeks gestation routinely undergo retinal examination at 38 weeks corrected age as part of ROP screening.ROP and retinal changes in premature babies are outside the scope of this study.

Retinal examination has been shown to be an uncomfortable procedure in babies (33). A literature review to determine the causes of pain and the best method of pain relief for babies undergoing retinal examination was performed. This literature review is included in this manuscript as a review article (33) and it was used as a guide in pain management for babies enrolled in this study.

The infants' eyes were dilated 45-60 minutes before the exam as per the neonatal department protocol (using 2.5% Phenylepherine, 0.5% Cyclopentolate (Alcon Laboratories Inc, Fort Worth, TX, USA)). After topical anaesthetic (Oxybuprocaine hydrochloride 0.4% (Bausch and Lomb, UK)) was applied, a lid speculum was put in place and a coupling gel applied to the camera's lens. The RetCam was placed gently on the eye and a posterior pole image was taken. Next the temporal and nasal retinal fields were imaged and then the superior and inferior retinal quadrants were captured. A neonatologist and a neonatal nurse carried out the procedure.

The most reliable images were obtained when the infant remained calm and had adequate papillary dilatation. The focus button on the control panel was used to obtain the clearest image. Still retinal images with optic disc in the centre were taken from both retinas. The sharpest image was then chosen for vessel measurement.



Figure 4. Retinal examination using the retinal camera. On the right is image of the retina taken with Retcam II (Clarity Medical System, Pleasanton, CA 94588, USA).

Retinal image analysis

Retinal image analysis was carried out on all retinal images taken from term infants.Measurements of vessel diameter were then obtained using semi-automated software (Vesselmap, IMEDOS GmbH, Jena, Germany) (38-40). The vessel diameter was computed as the distance between the walls within the vessel. The calibre of directly viewed vessels was determined by the size of the red cell column, because the vessel walls and peripheral plasma layer are nearly transparent. Vessels were measured in each eye, and the largest venule and arteriole of each patient was determined (38-40). Retinal artery branchpoints were not calculated because of technical difficulty.

Outcome measurements

Kidney volume and function at 32 weeks and at 38 weeks corrected age Kidney volume and function and retinal vessel measurement in term infants

Patients recruited for this study

The number of term infant admissions to Department of Neonatology at the completion of recruitment period was 524 neonates, of which 227 fulfilled the recruitment criteria. Consent was obtained from a parent of 43 infants. For analysis involving renal assessment, four patients had to be excluded (renal abnormalities), whereas five patients were excluded when data from kidney and eye assessments were needed (one patient excluded due to retinal haemorrhage). Twenty-four of the infants had a birth weight that was appropriate for gestational age, whereas 13 were LBW infants (Figure 5). In two of the original research articles (41, 42), data analyses were carried out using preliminary data during the recruitment period. For the remaining (43, 44), data analyses were carried out once recruitment was completed. A full description of the patients recruited and analysed is discussed in the respective publications.

A total of 288 premature infants were admitted to the neonatal intensive care unit during the study period. Forty-nine preterm babies, out of 112 who fulfilled the recruitment criteria, were recruited; 2 died and 3 were transferred back to regional hospitals, which made them unavailable for assessment. Therefore, data from 44 preterm babies were used for analysis (18 female, 26 male) (Figure 6).

Term infants



Figure 5. Breakdown of term babies admitted and recruited for this study

Premature infants



Figure 6. Breakdown of premature babies admitted and recruited for this study

Chapter Three – Published Papers

Review articles

- Kandasamy Y, Smith R, Wright IMR. Retinal microvascular changes in low-birthweight babies have a link to future health. *Journal of Perinatal Medicine*. 2012; 40(3):209-14.
- Kandasamy Y, Smith R, Wright IMR. Oligonephropathy of Prematurity. *American Journal of Perinatology*. 2012; 29(2):115-20.
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Retinal microvascular changes in low-birth-weight babies have a link to future health

Yogavijayan Kandasamy, Roger Smith, and Ian MR Wright Journal of Perinatal Medicine. 2012; 40(3):209-14

Abstract

Background: *In utero* insults that result in low-birth-weight (LBW) infants are now recognized as risk factors for the development of vascular-related diseases in adulthood. Microcirculatory pathologies are believed to form a mechanistic link between fetal insult and the manifestation of illness in adulthood.

Objectives: The challenge has been to investigate microcirculatory changes *in vivo*. The objective of this review is to determine whether LBW infants and individuals undergo abnormal microvascular changes and, if so, whether these changes can be identified and measured objectively by investigating retinal vessels.

Methods: An online publication search was carried out using the following keywords to identify and review relevant articles: retinal microvasculature, retinal vessels, small for gestation age, growth restriction, and intrauterine growth restriction. Articles published from 1980 to 2011 were considered.

Conclusions: The ability of retinal imaging technology to assess and measure the retinal microvasculature makes this a valuable assessment tool. However, the current tool is unsuitable for noninvasive assessment in infants and young children. Once this hurdle has been overcome, a longitudinal study of LBW individuals from infancy to adulthood, with regular retinal microvascular assessments, would help prove the mechanistic link between LBW and cardiovascular disease in adulthood.

Keywords: Low birth weight, retina, microvascular, microcirculation, retinal vessels

Introduction

Globally, more than 20 million low-birth-weight (LBW) infants are born each year [1]. The World Health Organization has defined LBW as a birth weight of less than 2500 g (5.5 lb) [2]. This is based on epidemiological observations that infants weighing less than 2500 g are approximately 20-fold more likely to die than heavier babies [1]. More common in developing than it is in developed countries, a birth weight below 2500 g contributes to a range of poor health outcomes. LBW defines a heterogeneous group of infants: some are born early, some are born growth-restricted (weight below the 10th percentile for their gestational age), and others are born both early and growth-restricted. It is generally recognized that a LBW is a disadvantage for the baby. *In utero* insults that result in LBW are now recognized as risk factors that contribute to the development of vascular-related diseases in adulthood [3–7]. The exact mechanism of this phenomenon has yet to be fully understood, but there is increasing evidence to suggest that microcirculatory pathology forms the mechanistic link between fetal insult and the manifestation of illness in adulthood [8–12].

The ability to investigate microvascular structure and function is important for improving our understanding of the mechanism of cardiovascular disease. The challenge has been to investigate microcirculatory changes *in vivo*. Various techniques are emerging for investigating different aspects of microcirculation in animals and humans [13]. Understanding the changes that occur in microcirculation may allow the identification of paths for early intervention in order to prevent adverse long-term consequences. Screening procedures that assess microcirculation may also allow the early identification of individuals at a high risk of cardiovascular and renal disease, thereby enabling early intervention in order to mitigate the effects of microvascular changes on long-term health.

The objectives of this review are to determine whether LBW infants and individuals have abnormal microvascular changes and whether these changes can be identified and measured objectively by investigating retinal vessels.

Methods

An online publication search was carried out using the following keywords to identify and review relevant articles: retinal microvasculature, retinal vessels, small for gestation age, growth restriction, and intrauterine growth restriction. Articles published from 1980 to 2011 were considered.

Human retina and microcirculation

The human retina has long been recognized as a site where human microcirculation can be visualized and assessed. In 1851, Hermann von Helmholtz presented his now-famous and classic monograph *Augenspiegel*, which made available to the world the technology to visualize retinal microcirculation [14]. Approximately 40 years later in 1892, Marcus Gunn [15] first described the changes in retinal vessels that occur in hypertension. It thus became recognized that the human retina provides a window for the *in vivo* investigation of human microcirculation. The availability of digital photography and the technology to store, retrieve, and transmit these data have made retinal imaging an important clinical tool [16].

The value of this imaging technique has been further enhanced by the availability of software to carry out automated analysis [17]. Retinal vascular analysis is currently used in two broad areas of cardiovascular research [16]. Firstly, it is a noninvasive research tool for investigating the pathophysiology and role of the microvasculature, typically defined as vessels between 100 and 300 μ m in size, in the development of clinical cardiovascular pathologies. Secondly, retinal vascular imaging is being explored in clinical settings as a risk stratification tool for helping clinicians identify patients with microvascular signs who are at a high risk of future clinical cerebrovascular and cardiovascular disease [16].

The automated diagnosis of retinal images using digital image analysis offers huge potential benefits in terms of the accuracy and speed of analysis [9, 16, 18]. There are various methods for image analysis. In a commonly used method, image analysis software identifies the retinal vessels that completely pass through the region between 1/2 and 1 disc diameter from the optic disc margin (zone B) and denotes their edges using the pixel density histogram technique [16, 17, 19]. The analysis is usually carried out on a sequence of complete trees, generally from a single eye but continuing onto the other eye if necessary in order to achieve the required number of vessel segments and bifurcations. The cross-sectional diameters of retinal arterioles and venules are measured repeatedly, and these measurements are then summarized using mathematical formulae in order to obtain values representing the mean arteriolar and venular caliber

of that particular eye. Other programmes such as ROPtool [20], Retinal Image Multiscale Analysis [21], and Computer-Aided Image Analysis of the Retina [22] use different techniques to measure retinal vessel dilatation and tortuosity semi-automatically.

Blood flow in vessels is not a random occurrence but rather it follows the principles of fluid mechanics in order to ensure it adheres to optimality principles. This was elegantly described approximately 85 years ago by Murray [23]. A vascular network can be defined in terms of bifurcation angles and junction exponents (a measure of the relative diameters of the parent and daughter branch vessels) at branching points. These parameters have implications on circulatory energy costs [24] and the efficiency of space filling by vascular networks [25]. Various types of circulation processes in humans conform to optimal health patterns, including the retinal microvascular architecture [25, 26]. In adults, aging and possibly hypertension are associated with a disadvantageous branching geometry, such as narrower bifurcation angles and smaller vessel diameters in the human retinal vasculature [27]. These findings imply increased power costs for blood transport and the uneven distribution of shear forces throughout the microvascular tree [23].

Retinal microvascular changes in LBW adults and children

Do LBW patients undergo microcirculatory changes and, if so, can these changes be detected through the assessment of the retinal microvasculature? Chapman et al. [28] investigated the association between LBW and alterations in the retinal microvasculature. In their study, a random sample of 100 men aged 64–74 years was selected from a cohort of men whose birth weights were known. They were stratified by birth weight (low \leq 3200 g or high \geq 3700 g) and systolic blood pressure (low \leq 140 mmHg or high \geq 160 mmHg) to yield four groups: LBW and low blood pressure, LBW and high blood pressure, high birth weight and low blood pressure, and high birth weight and high blood pressure.

Retinal photographic images were taken for each adult patient by an investigator who was blinded to the birth weight and clinical status of each participant. These photographs were converted into digital images using a special camera (Micro-Nikkor 60 mm f/2.8D lens, SWK-21-CCd camera, Nikon, Tokyo, Japan) and a personal computer (Viglen, Alperton, Middlesex, UK). These images were measured, and vessel

diameters and branching angles were calculated using commercially available software, namely Java (Jandel Scientific, Corte Madera, California, USA) and Mathcad (MathSoft Inc., Cambridge, Massachusetts, USA).

The investigators found that the LBW groups had significantly narrower arteriole bifurcation angles than did the high-birth-weight groups. The investigators postulated that narrower arteriole bifurcation angles are associated with increased circulatory energy costs and that this may be related to a lower than normal microvascular density. They concluded that differences in retinal microvascular architecture might reflect a persistent alteration in the vascular architecture as a result of an impairment during fetal development and suggested that this could provide a mechanistic link between LBW and increased cardiovascular risk.

In another study, retinal vessel morphology was evaluated in 21 young adults who were born small for gestational age (SGA) [29]. Digital images were compared with those taken from a group of 23 subjects born at an appropriate gestational age (AGA). The investigators found that SGA subjects had significantly less retinal vascularization, as evidenced by the lower number of vascular branching points (median: 26; range: 20–31) compared with AGA subjects (median: 28; range: 26–32) (Figure 1). They concluded that intrauterine growth restriction is associated with abnormal retinal vascular morphology in young adult life. They proposed that this finding may not be restricted to the retina and might represent a more global effect of vascular growth within the body.

Liew at al. [10] examined the association of birth weight and retinal arteriolar caliber in 3800 subjects aged 51–72 years in four US communities. Participants born term with known birth weights underwent retinal photography. Retinal arteriolar and venular calibers were measured from digitized retinal photographs by using a validated computer-assisted method. The investigators found that a lower birth weight was associated with narrower retinal arteriolar calibers, because each kilogram decrease in birth weight was associated with a 2.4 μ m (95% confidence interval (CI), 1.3–3.5; *p* < 0.001) narrowing of retinal arteriolar calibers after controlling for age, gender, race, education, smoking, alcohol consumption, adult body mass index, and height.

There is evidence that retinal microvascular changes are detectable from childhood in LBW individuals. Mitchell et al. [30] examined the relationship between birth parameters and the retinal microvasculature in 1369 six-year-old children in Sydney, Australia. Birth weight, birth length, and head circumference were obtained from parental records. Retinal arteriolar and venular calibers were measured from digitized retinal photographs by using a previously published protocol [31]. The investigators found that lower birth weight, shorter birth length, and smaller head circumference were associated with narrower retinal arteriolar calibers (Figure 2). Each kilogram decrease in birth weight was associated with a 2.3 μ m (95% CI, 0.6–3.9; *p* < 0.01) narrowing of retinal arteriolar calibers after controlling for age, gender, ethnicity, height, body mass index, axial length, mean arterial blood pressure, and prematurity. Similar associations with narrower retinal arteriolar calibers were observed for shorter birth length and smaller head circumference. The authors concluded that children who had lower birth weights, shorter birth lengths, and smaller head circumferences had narrower retinal arteriolar calibers.

In another study of the relationship between the retinal microvasculature and birth weight, 263 children were systematically screened in their 12th years of follow-up [12, 32]. Complete data were available for 166 children with gestations of \geq 37 weeks. The children were divided into three groups based on their birth weights (<3.2, 3.2–3.6, and >3.6 kg). Retinal circulatory measures were evaluated, including retinal microvascular tortuosity and bifurcation optimality deviance. The relationship between arteriolar diameters at a bifurcation is related to fluid power losses and endothelial function [23]. This relationship can be quantified by calculating an optimality ratio and the optimality deviation, which measure the extent to which the optimality ratio deviates from the theoretically predicted optimum [23, 33]. The optimality ratio is the sum of "daughter" arteriolar diameters divided by the "parent" arteriolar diameter. For a theoretically optimal bifurcation, the optimality ratio yields a value of 0.79 [33]. Departures from the theoretically predicted optimum are associated with increased power losses at bifurcations and are indicative of endothelial dysfunction [23, 32, 33]. Linear regression modeling was used to assess the association of retinal microvasculature measures with birth weight. The investigators found that optimality deviance (an indicator of endothelial dysfunction) and retinal tortuosity were higher among LBW children.

An Australian study also reported an association between smaller birth size and narrower retinal vascular calibers [34]. In total, 266 twins (49 monozygotic and 84 dizygotic pairs; mean age 9.3 years) underwent ophthalmic examinations including the
use of retinal photography [35]. The investigators used linear regression models to determine the association between birth parameters and retinal vessel diameter and found that each kilogram decrease in mean birth weight was associated with a 4.53 μ m narrowing of mean retinal arteriolar calibers. Furthermore, a 5 cm decrease in mean birth length was associated with a 4.79 μ m narrowing of mean retinal arteriolar calibers and each 2 cm decrease in head circumference at birth was associated with a 2.68 μ m narrowing of mean retinal arteriolar calibers. However, there were no overall statistically significant associations between these birth parameters and retinal venular calibers.

Retinal microcirculation and systemic disease

Does an abnormal retinal microvasculature increase cardiovascular risk? McGeechan et al. [36] published a systematic review of the studies that have investigated the relationship between retinal vessel calibers and coronary artery while adjusting for traditional risk factors. A total of 21,428 participants who underwent retinal assessments were followed over time (median follow-up period: 4.9 to 14.5 years) and monitored for cardiovascular disease events. Retinal photographs (film or digital) for one or both eyes, centered on the optic disc and macula, were taken. These photographs were then viewed by trained graders masked to participant characteristics, who measured the diameters of all arterioles and venules. The investigators demonstrated that variations in retinal vessel calibers (both wider retinal venules and narrower retinal arterioles) were associated with an increased risk of incident cardiovascular disease. The risk associated with changes in retinal vessel calibers was higher among women without diabetes or hypertension.

Other studies have demonstrated an association between retinal microvascular changes and stroke/neurological function [37–41], renal diseases [42–45], and diabetes mellitus [45–47].

Role of perinatologists and neonatologists: should screening begin in infancy?

Do LBW infants have abnormal retinal microvasculatures from infancy? To date, this question remains to be fully understood. Normal retinal microvasculature measurements in term infants have only become available recently [48]. Although the retinal microvasculatures of premature infants are routinely assessed to detect and treat

retinopathy of prematurity (ROP) [49], LBW infants, particularly SGA term infants, rarely fulfill the ROP screening criteria (i.e. infants < 1500 g, gestation < 32 weeks) and thereby they do not routinely undergo eye assessments. These infants do not receive regular follow-ups, unlike premature infants [50]. Moreover, there is no consensus on following up to assess neurodevelopmental outcomes for SGA infants.

It is important that physicians who care for SGA infants appreciate that this group of individuals has a higher risk of developing cardiovascular-related diseases. A longterm follow-up, similar to those that are recommended for ex-premature infants, would be helpful to monitor the health statuses of these infants as they grow. Once such follow-ups become available, the option of having infants undergo serial retinal vascular assessments can be seriously considered. However, technology hurdles have to first be overcome before serial retinal assessments can become standard practice in follow-up clinics.

The retinal cameras that are currently used on neonates are unsuitable for older infants. The ability of infants to keep their eyes still very briefly while undergoing eye assessments also makes this a challenging procedure [48]. Another possible difficulty in assessing the retinal microvasculature is our inability to account for refractive errors that could influence measurements [51]. In adults' and children's eyes, corrections can be applied to compensate for inaccuracies in the measurement of the retinal structure that occur because of refractive errors; these corrections require ocular biometric measurements, including axial length, anterior chamber depth, lens thickness, vitreous chamber depth, and keratometry (measurements of the curvature of the anterior surface of the cornea) [52]. However, these calculations are more challenging in infants because of the continued growth of their eyes [52]. Newer and more portable technology for retinal imaging is becoming increasingly available, and it is likely in the near future that such devices can be used to capture retinal images in young infants [53].

Conclusion

Retinal microvascular changes in LBW infants can be used as a risk stratification tool for future cardiovascular disease. Current research has proven that LBW individuals have abnormal retinal microvasculatures compared with the average population. There is sufficient evidence to show the association between changes in the retinal microvasculature and cardiovascular disease. Future research could take the form of a longitudinal study of LBW individuals from infancy, with regular retinal microvascular assessments using new technology to demonstrate the mechanistic link between LBW and cardiovascular disease in adulthood. Such a study, which would detect early microvascular changes noninvasively through the serial assessments of retinal microvasculatures in LBW individuals, could improve our understanding, and perhaps even explain Barker's hypothesis.

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Figure 1. Number of retinal branching points in subjects with IUGR ($n = 21; \bullet$) and in subjects who were born AGA ($n = 23; \circ$) in relation to a healthy reference group of control subjects. The upper dotted line depicts the 5th centile and the lower dotted line depicts the 95th centile range, and the solid centerline indicates the median in relation to percentage of birth weight deviation from the mean of the normal population (reproduced with permission [29]).



Figure 2. Relationship between birth weight and retinal arteriolar/venular caliber (reproduced with permission [30])

Oligonephropathy of Prematurity

Yogavijayan Kandasamy, Roger Smith, and Ian MR Wright

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Abstract

Objectives: With improved health care, the number of premature babies who survive to adulthood is expected to increase. The objective of this review is to determine whether premature infants have an increased risk of chronic kidney disease (CKD).

Study design: A literature review was performed by searching PubMed (US National Library of Medicine) and the Cochrane Library, using the keywords prematurity, kidney, nephrogenesis, oligonephropathy, and kidney impairment. Articles published in English since 1990 were reviewed.

Results: Increasing evidence suggests that prematurity causes oligonephropathy independently of, and coexisting with, intrauterine growth restriction. Animal studies show that nephrogenesis continues for up to 3 weeks in extrauterine life, but with up to 18% abnormal glomeruli. Nephrogenesis is further impaired in preterm infants who develop renal impairment in the early postnatal period, which is estimated to be 8% to 24%.

Conclusion: Premature infants are at risk for CKD. A larger longitudinal study is needed that follows up premature infants to determine the exact incidence of CKD. Until then, renal assessment in ex-premature infants should be incorporated into follow-up guidelines, in addition to the current assessment of growth and neurodevelopmental outcomes. The cost implications to a comprehensive program, impact of early identification and strategies to improve outcomes in this population are needed.

Keywords: oligonephropathy, preterm, nephrogenesis, chronic kidney disease

Introduction

The World Health Organization estimates that approximately 12 million premature babies are born per year and the numbers are increasing.¹ Current knowledge suggests that low birth weight (LBW; < 2500 g)² constitutes a risk factor for adult renal disease.³⁻⁶ LBW is associated with a lower nephron number, and individuals with LBW are at greater risk of developing chronic kidney disease (CKD) in adult life than are individuals with normal birth weight.⁵⁻⁹ LBW infants constitute a heterogeneous group of babies who can be premature, growth restricted (birth weight below the 10th centile), or both. This literature review presents data to support the association of prematurity and the development of CKD.¹⁰⁻¹²

Embryology

Kidney development starts early in gestation, with the pronephros appearing at approximately 3 weeks of gestation, followed by involution by 5 weeks, as the mesonephros and metanephros develop.¹³ Although the mesonephros degenerates by approximately the 11th week, the metanephros continues to develop until weeks 34 to 36, when nephrogenesis is complete.^{14,15} Nephron maturation begins in the juxtamedullary region and then progresses outward to the capsule.¹⁶ The critical window of the final stages of kidney development thus spans 32 to 35 weeks of gestational age (GA), and no new nephrons are formed after 36 weeks GA. Nephron endowment is, at that point, fixed for life.^{4,15} Premature infants are born prior to these final stages and so risk incomplete nephrogenesis. Occasionally, a rare genetic condition such as renal coloboma syndrome, an autosomal dominant disorder caused by PAX2 gene mutations, interrupts normal kidney development and causes renal hypoplasia.¹⁷

Animal studies

Animal studies have shown that insults during the development of the fetus could have a deleterious effect on the growing kidneys. Because of the complexity of the postnatal care of the premature human infant and the possible confounding factors associated with human autopsy studies, it is essential to undertake controlled animal studies in an appropriate model to examine the effects of preterm birth on nephrogenesis.¹⁸ The kidney in a baboon matures just before birth, similar to that of the human fetus, making

the baboon fetus the most suitable animal experimental model to investigate the impact of in utero insult on kidney growth.¹⁸ Gubhaju et al.¹² showed that in prematurely born fetal baboons, glomerular and nephron numbers increased in the first 3 weeks of extrauterine life during which period the fetuses continue to receive respiratory support in the neonatal intensive care. Similar to findings in preterm human neonates,¹⁹ kidney weight and volume relative to body weight were higher in the premature baboon neonates compared with GA-matched controls, suggesting an increased demand on the neonatal kidney following the transition from the intrauterine to the extrauterine environment. Although nephrogenesis was found to continue in the extrauterine environment following preterm birth, there was a high percentage (up to 18%) of abnormal glomeruli.^{12,20} It is, however, impossible to determine whether these changes are reversible, as the animals were euthanized after the renal biopsy was completed. Human preterm babies very often remain longer in a neonatal intensive care unit, undergoing various forms of treatment and intervention.

Animal studies involving other species have also shown adverse effects of commonly used medications such as aminoglycosides and steroids on fetal kidney growth.^{21,22} Antenatal exposure of gentamicin has been associated with reduction in nephron number.²³ The use of antenatal steroids has a huge impact on the outcome of premature babies.²⁴ In addition, animal studies have shown that fetuses exposed to antenatal steroids have a reduced number of nephrons.²²

Histopathological findings in humans

Rodriguez et al.²⁵ looked for evidence of abnormal postnatal glomerulogenesis in extremely LBW preterm infants. Renal autopsy tissues from 56 extremely premature and 10 full-term infants were studied. All preterm infants weighed less than 1000 g at birth and 42 (75%) of them were appropriate for gestational age (AGA). The GA ranged from 23 to 30 weeks. Preterm infants were divided into two groups (short survival of < 40 days and long survival of \ge 40 days). Each group was subdivided into those with acute kidney injury (AKI) and those with normal renal function. Using computer-assisted glomerular morphometry and radial glomerular counts (RGCs), the investigators showed that glomerulogenesis was markedly decreased in all preterm infants compared with term controls, the degree of which correlated significantly with GA. RGC is a technique in which layers of glomeruli are counted following a straight

line beginning in the deepest zone of the cortex, progressing systematically to the renal capsule. Although nephrogenesis continued after birth in the preterm infants, fewer glomeruli were produced. Preterm infants who developed AKI had significantly lower RGCs, suggesting that an insult in the early postnatal period further impaired nephrogenesis. The investigators concluded that prematurity itself could cause oligonephropathy.

A similar recent study investigated postnatal nephrogenesis in a cohort of preterm infants and fetuses.²⁶ Kidney samples were obtained at autopsy from eight human fetuses (15 to 22 weeks), 12 premature infants (GA ranging from 25 to 38 weeks), and three term newborns. None of the deaths were related to renal conditions. Eight of the 12 premature infants weighed less than 1000 g and two were less than 2500 g. In each kidney, RGCs were determined similarly to the earlier study,²⁵ and the investigators found that in those infants who were born alive, nephrogenesis continued for only a short period after birth (up to 3 weeks). The renal autopsy from a preterm infant who survived the longest in this cohort (3 months) showed retardation in glomerulogenesis despite an increase in body weight.

Both of these studies provide histopathological evidence that premature babies could have impaired postnatal nephrogenesis. Babies who are born prematurely end up with a nephron deficit from infancy. Lower nephron endowment is believed to increase the risk of hypertension and chronic renal diseases in later life.^{9,27} Glomerular filtration rate (GFR) is determined by the filtration rate of a single nephron and by the total number of nephrons. When the number of nephrons is diminished, the kidney compensates by increasing the single-nephron GFR.²⁸ According to the Brenner's hypothesis, this compensatory mechanism of single-nephron hyperfiltration leads to proteinuria, hypertension, glomerulosclerosis and ultimately CKD.²⁹

Clinical studies

With improved standards of clinical care and technology, more and more premature infants are being saved. A premature baby born at 24 weeks gestation can be expected to stay in the neonatal intensive care unit for 16 to 18 weeks or longer.³⁰ During this period, the infant will be subjected to various forms of invasive procedures and treated with various medications, some of which have been recognized to cause renal impairment.²⁸ The incidence of premature infants developing AKI during this period is

high.³¹ AKI is a complex clinical condition of the kidney ranging from mild dysfunction to complete renal shutdown with anuria.²⁸ Due to the shortcomings of using serum creatinine-based formulas to describe AKI, the exact incidence of AKI in neonates is unknown. Nonetheless, reported estimates are between 8 and 24%.³¹

Studies in very low birth weight (VLBW) infants have shown that the presence of AKI and elevated serum creatinine are independent risk factors for mortality.³² Neonates with AKI are believed to be at higher risk of developing CKD.²⁸ Longitudinal follow-up data of pediatric patients after AKI irrespective of cause showed that this cohort of children has a high risk of ongoing residual renal injury and death in the long run.

Huang et al.¹⁹ investigated early postnatal kidney growth in premature infants, comparing intrauterine and extrauterine renal growth. One hundred neonates were enrolled in the study and renal volumes were measured by ultrasound. Extrauterine renal growth in a group of premature infants who were less than 34 weeks was compared with intrauterine renal growth for a group of term infants. Left kidney volume, body weight, height, and age were used in the correlation analysis. Premature babies had smaller kidney volumes compared with their term counterparts at a similar GA. The authors concluded that kidney growth in premature babies was reduced compared with intrauterine renal growth at an equivalent GA.

Renal size has been measured in a cohort of young adults who were born prematurely. The patients recruited in this study were part of the Project on Premature and Small for Gestational Age Infants (POPS) cohort.³³ Subjects who were born prematurely (< 32 weeks GA) were divided into two groups (AGA and SGA). Babies born full term and AGA were recruited as controls. Kidney length and volume measurements were taken and then compared between each group and the controls. Renal size did not differ between SGA and AGA individuals. Left kidney length and volume were significantly lower than right in both SGA and AGA individuals, notably more so in women. The left kidney was larger than the right one in 70% of controls compared with 40.9% of the SGA group and 48.3% of the AGA group. Renal structural anomalies were present in only eight prematurely born participants. The investigators concluded that kidney growth is stunted after preterm birth, especially on the left side, and more so in females. The long-term consequences of this association, however, have not been determined.

Others have evaluated large cohorts of children with CKD to determine perinatal demographics (such as SGA, LBW) with CKD. Franke et al.³ reviewed perinatal parameters of 435 children with CKD. Kidney disease was classified as either congenital with onset of renal disease during fetal life (n = 260; 60%), hereditary as genetically determined with onset after 3 months of life (n = 93; 21%), or acquired (n =82; 19%). The investigators found that the rate of prematurity was elevated in children with congenital (39.3%), hereditary (24.7%), and acquired CKD (15.5%) compared with the rate of prematurity in children with normal renal function (8%); similarly, the rate of small for gestational age (SGA) babies was elevated in children with congenital (29.2%), hereditary (22.6%) and acquired CKD (29.3%). The investigators concluded that both SGA and prematurity predispose a child to CKD. Greenbaum et al.³⁴ investigated the association between abnormal birth history and growth in children with CKD. In this study, growth outcomes from 426 participants were assessed. This cohort had a high prevalence of LBW, SGA, and premature infants. In this study, growth outcomes were quantified by age-sex-specific height and weight z-scores during clinical visits. This study showed that patients who were born with LBW and SGA had poorer growth and weight gain. Incomplete nephrogenesis could lead to persistent oligonephropathy, which therefore may represent a major risk factor for progressive renal disease in adulthood.

Is there any evidence that oligonephropathy can be seen in adults born prematurely? Focal segmental glomerulosclerosis (FSGS) is recognized as a pattern of injury mediated by elevated glomerular capillary pressures and flow rates. These changes can occur as an adaptive response in conditions in which the number of functioning nephrons is reduced.³⁵ Hodgin et al.³⁶ reviewed histopathological data from six adults who were born at 22 to 30 weeks gestation with a mean birth weight of 1054 g (range 450 to 1420 g). These patients all had clinically significant proteinuria and no other potential risk factors for secondary FSGS. A review of the renal biopsy results showed that these patients had histopathological changes typical of postadaptive FSGS (glomerulosclerosis, glomerulomegaly, and mild foot process effacements). The investigators concluded that prematurity promotes the development of secondary FSGS and, because birth history is often omitted by physicians who treat adults, this risk factor is likely to be underrecognized. This study is limited by the number of patients reviewed and by the fact that neonatal care has changed tremendously in the last 20 years.

Follow-up of ex-premature infants

Should infants who were born prematurely thus be screened regularly for evidence of renal impairment? The currently available guidelines in the United Kingdom recommend that the assessment of health status at two years of age, corrected for prematurity, be carried out for all births of less than 31 weeks gestation or less than a birth weight of 1000 g.³⁷ The focus of this guidelines is on assessment of growth, neurodevelopmental outcome, and cognitive function. No specific recommendations exist regarding assessment of renal status (blood pressure, renal function, or urine analysis) when the child is seen at two years of age. Similarly, long-term follow-up plans from other countries focus on developmental outcomes and the incidence of chronic lung disease.^{38,39} There is no consensus about how renal assessment should be done on premature infants upon discharge. It has been proposed that blood pressure measurements should initially be monitored yearly and, later, every two to three years for at least two or three decades in all people born prematurely.⁴⁰ This suggestion appears to be very practical, considering the earlier information. Evidence shows that young adults who were born prematurely have elevated blood pressure.⁴¹ Blood pressure in a cohort of 50 young adults who were born prematurely (< 32 weeks gestation; 23 SGA and 29 AGA) was compared with that from a group born at term. Twenty-four-hour ambulatory blood pressures and renin concentrations were determined in all subjects. Systolic blood pressure in both AGA and SGA premature infants was higher than in control infants. No significant difference was observed in the blood pressure of AGA and SGA infants. The renin level in the AGA group was found to be higher, although the clinical significance of this finding is unclear. The authors concluded that very preterm individuals have higher systolic blood pressures as well as a higher risk of hypertension at a young adult age.

Bacchetta et al.¹⁰ performed an assessment of renal function on a group of 50 children who were born preterm (GA < 30 weeks). A group of babies born at term were recruited as the control group. The mean age in this cohort was 7.6 years. Blood pressure, kidney size, and GFR using inulin clearance were measured in both groups. Children who were born preterm had smaller kidneys, elevated mean diastolic blood pressures, and impaired GFR. As a result, the authors recommended that long-term renal follow-up (blood pressure, serum creatinine, urine albumin-to-creatinine ratio) be performed on all children who are born preterm. This study, however, did not provide

any data on neonatal management (such as umbilical artery catheterization) and its possible influence on blood pressure.

The role of routine urine analysis in the detection and prevention of CKD is not well defined. Ambiguity about screening children exists because of the uncertainty as to whether early detection of renal disorders in childhood will lead to effective interventions and a reduction in the number of individuals who subsequently progress to CKD. A related concern is whether the adoption of urinary screening programs is cost-effective. The most common method used for screening children for CKD involves the measurement of spot samples of urine for hematuria and/or proteinuria.⁴² A subtle increase in urinary albumin excretion, known as microalbuminuria, has been identified as a prognostic marker for cardiovascular and/or renal disease in diabetic and nondiabetic adults.⁴³ Microalbuminuria is defined as urinary albumin excretion from 30 to 300 mg/24 h or equivalent amounts (3 mg/mmol creatinine or 30 mg/g creatinine) when spot urine samples are used. For clinical purposes, immunologic techniques are most frequently used, as they are easy to use at relatively low cost. In the absence of any inflammation in the urinary tract, intact albumin of glomerular origin is the major source of albumin in the urine.⁴³

In a European study, spot urine tests from 109 young children who were in a cohort of VLBW (birth weight < 1000 g) were tested for microalbuminuria with the more sensitive technique of high-performance liquid chromatography (HPLC).⁴⁴ Results obtained with the conventional immunoassay technique were compared with those obtained by using HPLC. The percentage of patients diagnosed with microalbuminuria was four times greater with HPLC than with the conventional technique (44.5% vs. 11%, P<0.001). The investigators concluded that microalbuminuria in a VLBW child was more marked when HPLC was used and the difference was significant. The high cost and expertise required for HPLC means that it can currently only be used as a research tool and may not be suitable for routine follow-ups of premature infants.

A follow-up study in children who survived AKI recommended that, in view of the incidence of renal insufficiency and death among this cohort, these children should be screened regularly for signs of renal disease.⁴⁵ However, there is no consensus on how this assessment should be done.

Future research

The limitation of some of these studies^{33,36} is that they are unable to ascertain the number of patients with morphological changes (smaller kidneys) and histopathological changes who will develop symptomatic renal disease. Reduced kidney size has been proposed as a surrogate marker for reduced nephron mass, as the nephron number can only be determined by renal biopsy.²⁷ A lower nephron number has been associated with hypertension and renal diseases in adulthood.⁴⁶ Long-term studies are needed to determine the percentage of premature patients with hypertension, proteinuria, and abnormal renal function that develop CKD in later life. A cost-effectiveness study should also be conducted to determine the benefits of routine screening for renal conditions. These studies can also help determine the cost implications of these patients for the health care system, should they develop CKD. It costs more than US\$ 65,600 to treat an extremely premature infant (birth weight < 1000 g) and this figure is higher for the infants who develop other complications such as renal impairment.⁴⁷ CKD screening programs theoretically would allow for implementation of interventions in earlier stages, eventually decreasing costs because of less progression to CKD and improved health status at kidney replacement therapy initiation.⁴⁸ These follow-ups and assessments (blood pressure, urine for microalbuminuria, renal function, possibly using newer markers such as urinary neutrophil gelatinase-associated lipocalin) could be incorporated into the existing follow-up guideline.³⁷ In addition, the long-term impact of maternal perinatal condition and treatment on infant's health will need to be reviewed. Once the results from long-term studies are available, perhaps an intervention similar to those that have been successful in delaying CKD in adults can be considered from infancy.⁴⁹ A question that we can try to address after we have sufficient clinical data is, should nephron protection begin at birth?⁴

Conclusion

The number of premature births continues to increase, and with improved health care and technology, more of the infants will survive to adulthood. Therefore, the increased number of children and adults with incomplete nephrogenesis will contribute to a rise in the number of CKD patients in the future. Most studies include both AGA and SGA cohorts, but a large longitudinal study that follows AGA premature infants to determine the incidence of kidney disease is needed. The final effect of oligonephropathy is likely to be a combination of inadequate nephrogenesis due to premature birth, complicated by abnormal glomerulogenesis and AKI in the neonatal period. Mechanisms and guidelines have already been established for the follow-up of premature infants, which could be modified from their current focus on neurodevelopment outcome to also include routine renal assessment. Currently, it has not been well established whether renal assessment and treatment for ex-premature infants will prevent or delay the occurrence or presentation of renal disease. Future research should address all these aspects, including the incorporation of renal surveillance into the follow-up plans for preterm cohorts.

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Measuring Cystatin C to Determine Renal Function in Neonates

Yogavijayan Kandasamy, Roger Smith, Ian MR Wright Accepted for publication by *Pediatric Critical Care Medicine Journal* 2013;14(3):318-22

Abstract

Objective: The incidence of acute kidney injury (AKI) in neonates is high and associated with up to a 50% mortality rate. The purpose of this review was to determine the feasibility of using serum cystatin C (CysC) measurements to assist clinicians in making early and accurate diagnoses of AKI in neonates.

Data source: We searched for the following 7 key words within the PubMed database and The Cochrane Database of Systematic Reviews: Cystatin C, neonates, newborn, preterm, premature, kidney failure, and kidney injury.

Study selection: The selected studies included neonates within their study populations and were published in English. We reviewed literature published between January 1990 and May 2012.

Data extraction: Ten studies had conducted serum CysC measurements in neonates.

Data synthesis: The CysC level in neonates is not influenced by the maternal level and is highest at birth. In most studies, CysC levels on day 1 of life ranged between 1 and 2 mg/L, gradually declining during the first year, and then remained relatively stable thereafter. CysC levels did not differ between male and female infants, and no significant gestational age-dependent differences were found. CysC level were increased in cases of sepsis, AKI, and congenital renal abnormalities.

Conclusions: CysC has all of the theoretical properties needed to be an ideal marker of renal function. It can be used to determine base line renal function on day 1 and is increasingly being used to determine renal function in sick neonates. In the majority of studies, the day 1 CysC level ranged between 1 to 2 mg/L, which gradually declined in the first year of life. However, the number of available studies evaluating CysC in sick neonates is currently limited, and there are also no studies linking CysC levels in sick babies with short-term and long-term outcomes.

Keywords: creatinine, cystatin C, acute kidney injury, neonates, preterm, renal failure

Introduction

Acute kidney injury (AKI) is a complex clinical condition ranging from mild kidney dysfunction to complete renal failure with anuria (1). It is characterized by a decrease in the glomerular filtration rate (GFR), increase in the serum concentration of creatinine and nitrogenous waste products, and the inability of the kidney to maintain fluid and electrolyte homeostasis (2). AKI and elevated serum creatinine (SCr) levels are independent risk factors for mortality (3). Moreover, neonates with AKI are believed to be at a higher risk of developing chronic kidney disease (1). The incidence of AKI in neonates varies according to the underlying pathology. In one prospective study involving 229 very low birth weight babies, 18% of the cohort developed AKI, with a mortality rate of 42% (4). The incidence of AKI in neonates undergoing cardiac surgery is higher, with one study showing that approximately half of the patients developed AKI with a mortality rate that was 4 times higher than those without AKI (5). Similarly, a very high incidence rate of AKI (41.7%) has been reported in neonates with perinatal asphyxia (6). Importantly, our inability to recognize AKI has prevented early intervention and improved outcomes.

Measuring renal function in neonates

Creatinine clearance and SCr levels are routinely used to monitor adult renal function. However, the use of SCr to measure the GFR of neonates has never been completely investigated. Van Anker *et al.* evaluated 144 preterm infants (gestational age 23.4-36.9 weeks), where they measured GFR as the clearance of inulin, which was determined from the infusion rate and SCr level (7). They observed that the level of SCr was inversely proportional to the clearance of inulin and concluded that the reciprocal of the SCr value provides an accurate measurement of glomerular filtration (7). However, using SCr levels to determine GFR has many limitations. For example, assays that measure SCr levels are subject to negative interference by bilirubin and hemoglobin and positive interference by cephalosporin and ketones (7). In addition, other limitations include (i) SCr levels are not altered until there is a loss of 25-50% of kidney function; (ii) SCr levels overestimate renal function due to tubular secretion of creatinine at lower GFRs; and (iii) SCr levels are influenced by an infant's muscle bulk, hydration status, and gestational age (1). At birth, SCr concentrations reflect both infant and maternal levels (8). It was previously believed that the SCr concentration in newborns steadily declines after birth. However, recent data have shown that the SCr concentration increases during the first 48 hours of extrauterine life, subsequently reaches a peak, and then declines thereafter as the GFR rises (8). Because of incomplete nephrogenesis, the GFR in pre-term neonates is significantly lower than that in full-term infants and matures more slowly as well. In a full-term newborn, the GFR at birth is approximately 20 mL·min⁻¹/1.73 m², which doubles within the first 2 postnatal weeks (9). However, a delayed rise in SCr after the onset of renal injury makes it an unreliable marker; thus, the search for early AKI biomarkers has been a prominent research focus (10).

CysC—composed of 122 amino acids—is a cysteine proteinase inhibitor with a relative molecular mass of 13,250 Da (11). It is produced by a housekeeping gene expressed in all nucleated cells at a constant rate and is freely filtered at the glomerulus with no tubular secretion. Moreover, CysC is completely catabolized by the renal tubules, and therefore its plasma level is only dependent on the GFR (11). Meta-analyses have shown that CysC is superior to SCr for the estimation of the GFR in children (12, 13). Andersen *et al.* (13) evaluated the usefulness of CysC as marker for pediatric renal function based on literature on children from various age groups. They concluded that the sensitivity of serum CysC for detecting impaired GFR in the pediatric population is superior to that of plasma creatinine. However, the investigators did not make any comments or draw conclusions as to the value of CysC as a marker in neonates. Therefore, this review focuses on the current literature on CysC measurements in neonates and explores the possibility that this marker could substitute for creatinine measurements in clinical practice.

Methods

We performed a review of the literature from PubMed, US National Library of Medicine, and The Cochrane Database of Systematic Reviews, using the following keywords: Cystatin C, neonates, newborn, preterm, premature, kidney failure, and kidney injury. The keywords were searched alone or in combination with other keywords. The search was restricted to articles that had neonates in their study population and those that were published in English. We reviewed literature published between January 1990 and May 2012.

Results

To date, 10 studies on CysC measurements in neonates have been published (Tables 1 and 2) (14-23). Previous studies focused on determining the normal CysC reference range in term neonates and compared the value with maternal levels (14) and with older infants and children (15, 17). Subsequent publications compared CysC levels between premature and term babies (16, 18-20). More recent publications considered CysC as a marker for AKI in ill neonates (21-23). The measurements have been presented in various ways, including as a mean with 95% confidence interval, median and interquartile range (IQR), mean with one standard deviation (SD), and mean with 2 SD. The most recent studies have indicated that the CysC level in neonates does not have a normal Gaussian distribution, and have reported the measurements as a median with an IQR (20, 22, 23).

Method of analysis

Particle-enhanced immunonephelometry (PENIA) and particle-enhanced immunoturbimetry (PETIA) are the two most common methods used to measure serum CysC levels. The use of PETIA results in up to 30% higher CysC measurements (13), but recent studies have explored the use of the PENIA method. The International Federation of Clinical Chemistry and Laboratory Medicine/Institute for Reference Materials and Measurements Working Group for Standardization of CysC (24) are currently establishing a uniform calibrator for both techniques. This will facilitate a direct comparison of measurements obtained from both assays.

Normal CysC values

One of the earliest studies assessing CysC levels compared the levels in healthy women with their full-term neonates (14). Seventy-eight women with uncomplicated pregnancy and their newborns babies (43 males and 35 females) were enrolled in the study. The investigators evaluated the relationship between maternal and neonatal serum CysC levels and SCr levels. The gestational age ranged from 37 to 43 weeks and the birth weight ranged from 2.50 to 4.15 kg. Serum levels of CysC, creatinine, and urea were measured in all women immediately before delivery and in their newborns at the time of birth as well as 72 and 96 h after birth. The investigators found that the maternal serum CysC levels ranged from 0.64 to 2.30 mg/L at term gestation. At birth, the neonatal

serum CysC values ranged from 1.17 to 3.06 mg/L, and decreased significantly after 3-5 days of life. No correlation between maternal and neonatal serum CysC values was observed. The authors concluded that neonatal serum CysC originates exclusively in the neonate.

Rander *et al.* measured CysC levels in a mixed cohort of 12 neonates (age range 7 days to 1 month old) and 137 children (15). CysC levels were highest in the neonatal period and gradually declined from 1.63 ± 0.26 mg/L to a steady state of 0.51-0.95 mg/L for children aged at least 1 year. Two subsequent studies also compared CysC measurements between full-term infants and older children (16, 17), which corroborated the earlier finding of declining CysC levels during the first year of life. These studies also showed that CysC measurements do not differ between male and female infants. Subsequent studies showed that there were no gestational age-dependent differences in CysC measurements (16, 17, 20). Table 3 shows the comparison between SCr and CysC levels in neonates.

Cystatin C in ill neonates

The number of studies that have evaluated CysC as a marker for AKI in sick neonates is limited, and earlier studies have focused on determining CysC normal values. In the last 12 months, three studies that evaluated serum CysC measurements in different neonatal conditions have been published (21-23). Sarafidis *et al.* compared asphyxiated neonates (n = 13) with healthy neonates (n = 22) in a cohort study. In that study, a subgroup analysis was performed among babies with asphyxia where they were separated based on the presence or absence of AKI (n = 8 and n = 5, respectively) (23). A standardized definition was used to diagnose perinatal asphyxia and classify the severity according to Sarnat and Sarnat (25). AKI was defined as persistently increased SCr (\geq 1.5 mg/dL) for at least 24 h. In the asphyxiated group, serum CysC measurements were significantly higher, and SCr levels were also elevated. The CysC level on day one in babies with asphyxia was higher (3.03 vs. 2.86 mg/L, P < 0.05), and the level was reduced on the following days, which was attributed to improvement in renal function.

Maruniak-Chudek *et al.* compared serum CysC with SCr as a marker for renal function in 32 septic neonates (gestational age 34–40 weeks) admitted to neonatal intensive care during the first 2 weeks of life (21). The cohort was divided into 3 groups: sepsis (n = 9), severe sepsis (n = 14), and septic shock (n = 9). AKI was

determined using SCr and urinary output changes (26). Both CysC and SCr levels were higher in neonates with sepsis and severe sepsis. However, despite a difference in SCr between the 3 groups, there was no difference in CysC levels, and it was concluded that CysC does not offer any advantage over SCr in assessing renal function. The CysC level in sepsis, severe sepsis, and septic shock were 1.23, 1.47, and 1.50 mg/L, respectively, with a normal range of 0.81-2.6 mg/L. However, the authors cautioned that they had used a different measurement technique (ELISA) compared to the 2 commonly methods, and we were unable to determine how this method differs from PENIA and PETIA. Moreover, the main limitation of this study is the absence of control group.

Parvex *et al.* compared CysC levels between normal neonates and neonates with kidney failure secondary to congenital anomalies in order to determine residual renal function at birth for babies with congenital renal anomalies (22). Because maternal creatinine crosses the placenta, SCr is an unreliable marker for renal function at birth. Umbilical cord blood was collected from 100 term infants, and the median level with IQR was determined (median = 2.02 mg/L, IQR = 1.86-2.23). The investigators compared this level with that obtained from 33 term infants diagnosed antenatally with kidney anomalies, and this cohort was divided into 2 groups based on whether the anomaly was unilateral or bilateral. CysC was significantly increased by 24.5% (P < 0.001) in the cohort with bilateral kidney malformations compared to controls, independent of gender, weight, and size. Based on these findings, the investigators suggested that a high level of CysC in neonates with bilateral kidney malformation may reflect the low renal endowment in this cohort.

Conclusion

AKI is associated with an increased risk of morbidity and mortality in neonates, especially in very low-birth weight infants, infants with congenital heart disease who undergo cardiopulmonary bypass, and those with perinatal asphyxia. To improve outcome, AKI needs to be identified and managed early. Serum creatinine measurements have many inherent limitations, and there is sufficient evidence to suggest that SCr as a marker of renal injury results in a late and under-diagnosis of AKI. In contrast, CysC has all of the theoretical properties to be an ideal marker of renal function. Measurements can be easily taken through umbilical cord blood analysis, and there is sufficient information to predict the changes in the level that occur in the first

year of life. Earlier studies on CysC focused on determining the normal values in neonates. The number of studies evaluating CysC in sick neonates is limited at the moment, and there are no studies linking CysC levels in sick babies with short-term and long-term outcomes. In the majority of studies, the day 1 CysC level ranges between 1 and 2 mg/L, and this level gradually declines during the first year of life. Currently, there are insufficient data to determine the best level at which treatment should be initiated. Therefore, there is a need for more studies, especially in sick neonates with renal impairment. A few studies evaluating CysC in ill neonates have emerged more recently, which provides an encouraging trend. We postulate that there could be an increasing awareness among clinicians that CysC may have a role in the early detection of AKI . Therefore, we are hopeful that more studies will become available in the near future that can assist clinicians in the early diagnosis and treatment of AKI.

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 Table 1. Summary of studies of neonates and premature infants to determine the normal serum levels of CysC.

Study	Number of	Gestational age	Cystatin C level
	neonates	(weeks)	(Day 1)
			mg/L
Cataldi et al (1999)	88	Term	2.11 [1.45-2.81]^
Randers et al. (1999)	12	Term	1.63 ± 0.26 #
Finney et al. (2000)	16	24-28	1.48 (0.65–3.37) °
	14	29-36	1.65 (0.62-4.42) °
Harmoinen et al.	58	25-37	1.88 ± 0.36 #
(2000)	50	Term	1.7 ± 0.26 #
Treiber et al. (2006)	75	34-41	2.0 [1.38-3.23] ^
Armangil et al. (2008)	108	29.9-35.1	1.80 ± 0.3 #
Bariciak et al. (2011)	25	24-28	1.63 (1.17–2.24) ^
	35	28-32	1.79 (1.05–2.41) ^
	37	32-36	1.89 (0.58–2.93) ^
	27	> 36	1.84 (1.32–2.63) ^

Note : * = mean with 95% confidence interval; $^{\wedge}$ = median and interquartile

range (IQR); #= mean (SD); ° = mean (2SD).

Table 2. Summary of studies measuring Cystatin C level in ill neonates.

Study	Number of neonates	Gestational age	Cystatin C level
		(weeks)	(Day 1)
			(mg/L)
Maruniak-Chudek et	32	34-40	1.35 [1.20-1.49] *
al.(2012)			
Parvex et al. (2012)	20 (UKM)	37-42	1.88 [1.76–2.01]^
	13 (BKM)	37-42	2.52 [2.16–2.71]^
	100 (control)	40-41	2.02 [1.86-2.23] ^
Sarafidis et al. (2012)	8 (AKI)	38.2 ± 1.7	1.52 ± 0.43 #
	5 (no AKI)	37.2 ± 1.3	1.0 ± 0.18 #
	22 (control)	38.6 ± 1.1	$1.02 \pm 0.26 $ #

Note : * = mean with 95% confidence interval; $^{\sim}$ = median and interquartile range

(IQR); # = mean (SD); BKM = bilateral kidney malformation; UKM = unilateral kidney malformation; AKI = acute kidney injury.
Table 3. Comparison between Creatinine and Cystatin C levels in neonates.

Characteristics	Creatinine	Cystatin C
Influence from maternal plasma	Yes	No
level		
Level affected by gestational age	Yes	No
Level in the first week of life	Fluctuating	Gradual decline
Excretion by kidney	Yes	Yes
Reabsorption/secretion by renal	Yes	No
tubules		
Affected by infant muscle mass	Yes	No

Pain relief for premature babies during ophthalmology assessment

Y. Kandasamy, R. Smith, I.M.R. Wright, L. Hartley Journal of the American Association for Pediatric Ophthalmology and Strabismus (JAAPOS). 2011; 15(3):276-80

Abstract

Background: The ophthalmological examination of premature infants, which is essential for the detection of retinopathy of prematurity (ROP), can be painful and distressing for the infant. Various researchers have investigated the benefits of topical anesthesia, oral sucrose, and nonpharmalogical intervention for pain relief. The purpose of this study is to review the current state of knowledge on the effectiveness of these approaches.

Methods: A literature search was performed with MEDLINE (January 1980 to January 2011) and the Cochrane Central Register of Controlled Trials, Issue 1 of 4 (January 2011), to determine the currently available evidence on methods of pain relief for premature infants undergoing ROP examination.

Results: Most studies supported the use of topical proparacaine, which marginally decreased pain without any side effects. Oral sucrose did not significantly reduce pain scores during ROP examinations, and withholding feeding before the examination was not beneficial. Infants given pacifiers had lower pain scores than those without pacifiers, and infants who were nested experienced less distress during and after the procedure. Conflicting data existed on the benefits of different examination techniques, but the insertion of a lid speculum appeared to be the most uncomfortable aspect of the screening examination.

Conclusion: Topical anesthetics marginally reduce pain during eye examination in premature infants. Contrary to standard practice, it appears that patients are more comfortable if they are fed before the examination, and there is no benefit of oral sucrose. Nonpharmacological interventions, including sucking on a pacifier and nesting, may also be beneficial.

Background

Preterm birth, defined as childbirth occurring at less than 37 completed weeks or 259 days of gestation, is a major cause of neonatal mortality and morbidity and has longterm adverse consequences for health.¹⁻⁴ The World Health Organization estimates that there are more than 12 million preterm births per year and the numbers are increasing.³ A significant proportion of these babies will develop Retinopathy of Prematurity (ROP).^{5,6} The current joint policy statement on ROP screening produced by the American Academy of Pediatrics (AAP), American Academy of Ophthalmology and the American Association for Pediatric Ophthalmology and Strabismus recommends that babies with a birth weight of less than 1500 g or gestational age (GA) of 30 weeks or less and high risk infants undergo retinal examination to detect ROP.^{7,8} This guideline also recommends that efforts should be made to minimize discomfort and systemic effects of eye examination by pretreatment of the eyes with a topical anesthetic agent such as proparacaine and to use other methods such as pacifiers and oral sucrose although it does not provide any references to support these recommendations. Adequate pain relief is important in neonates. Neonates, unlike adults, are unable to express the amount of pain they are feeling. Inadequate pain relief may result in neonates developing increased pain sensitivity over time and altered responses to pain later in life.^{9,10} Animal studies on brain and spinal cord development suggest that repetitive pain during the neonatal period may cause permanent or long-term changes in the immature brain.^{11,12} This review assesses the currently available evidence on pain relief for premature infants during this uncomfortable procedure.

Neonatal Pain

Currently available evidence shows that ROP examination is a painful and distressing procedure for a premature baby.¹³⁻¹⁶ The neonatal pain management policy produced by AAP recognizes that ROP examination is a painful and uncomfortable procedure and cautions that the currently available methods of pain relief with topical anesthetic agents or oral sucrose may be insufficient.¹³ There are various methods which can be used to assess pain in babies.¹³ One of the more commonly used and established scoring methods developed to assess acute pain in preterm is the Premature Infant Pain Profile (PIPP) score.¹⁴ This is a 7-indicator composite measure that includes behavioral, physiologic and contextual indicators. PIPP consists of 3 behavioral (facial actions:

brow bulge, eye squeeze, and nasolabial furrow) and 2 physiological (heart rate and oxygen saturation) indicators, and 2 contextual (gestational age (GA) and behavioral state) variables that modify pain. Possible scores range from 1 to 21. PIPP scores <7 are indicative of no pain, PIPP scores 7 to 12 are indeterminate and >12 are indicative of significant pain. First introduced approximately 14 years ago, this tool has been validated in numerous reviews and continues to be a reliable method of assessing pain in preterm neonates.¹⁵ The AAP recommends that whatever pain assessment tools are used, continual multidisciplinary training of staff in the recognition of neonatal pain and in the use of the chosen pain-assessment tools should be provided.¹³

Topical Anesthesia

Topical anesthesia such as proparacaine hydrochloride is often used as a pre-treatment for the eyes prior to examination. This is a rapidly acting local anesthetic agent which has duration of action of 10 to 20 minutes. The efficacy of topical anesthesia during ROP screening has been rather controversial. Table 1 compares four different studies on the effects of topical anesthetic agent on pain relief in premature infants during ROP examination. All studies used proparacaine as topical anesthesia. In an earlier study by Saunder et al., 55 patients were recruited but the data was only available for 42 patients.¹⁶ No standardized method of pain scoring was used. Instead, patient's vital signs and cry intensity was recorded. The investigators found that there was no difference in any of these parameters between the two patient groups and concluded that topical anesthetic agents offered no advantage over normal saline eye drops during the examination of premature infants.

Subsequent clinical studies, however, have shown that the use of a topical anesthetic agent does have a significant effect on pain relief in premature infants. The authors, however, differed in their conclusions. A study by Marsh et al. concluded that topical anesthetic pretreatment with proparacaine during speculum insertion significantly reduces the PIPP score compared with saline and the authors proposed it should become routine practice.¹⁷ In a study by Mehta et al., the investigators suggested that proparacaine eye drops should be routinely used for the short-term, immediate relief of pain during ROP screening in preterm infants of lesser GA. In this study, the patients underwent assessment at GA 33.3 and 35.3 weeks.¹⁸ There was significant difference in PIPP scoring during the first examination between the treatment and

control group. However, PIPP scores at 1 and 5 minutes during the 2nd examination were significantly lower compared to first examination regardless of type of drops instilled (1 minute 11.9 vs. 10.15, P = 0.03 and at 5 minutes 5.7 vs. 4.475, P = 0.025). Mature infants felt less pain and tolerated ROP screening better. These investigators concluded that proparacaine is not beneficial for a mature infant. In a more recent study, Cogen at al. investigated the benefit of proparacaine compared to artificial tears during ROP eye examination.¹⁹ PIPP score was measured during the assessment of right eyes on three separate occasion (speculum insertion, indirect ophthalmoscopy and indirect ophthalmoscopy with scleral depression). Corneal clarity was subjectively assessed by the examiner. There was no difference in PIPP score between treatment and control group on all three phases of examination (P > 0.05). PIPP score was highest during scleral indentation in both groups but the significance of this was not determined in this study. Although the individual percentage of PIPP score being ≥ 11 was not different between treatment and control groups, the final cumulative comparison showed 65% of the examination in the control group had a score of ≥ 11 compared to only 27% in the treatment group (P = 0.04). There was no effect on corneal clarity. The authors concluded that topical anesthesia can marginally decrease pain in premature infants without any adverse effect.

Oral sucrose

Non-pharmacological agents such as oral sucrose are being used for pain relief in neonates. A Cochrane systematic review on sucrose for analgesia for in newborn infants undergoing painful procedures concluded that sucrose is safe and effective for reducing procedural pain.²⁰ The majority of the studies reviewed involved the effect of sucrose as pain relief in babies who had their heel lanced. However, an optimal dose could not be identified due to inconsistency in effective sucrose dosage among studies (ranges between 12 to 50% sucrose). This review analyzed 44 studies enrolling 3,496 infants. The authors also carried out a systematic review of five published articles on the effect of sucrose during ROP assessment in premature infants which are summarized in Table 2.²¹⁻²⁵ The overall pooled results, however, showed that oral sucrose did not cause a significant reduction in PIPP score in premature infants undergoing eye ROP assessment (weighted mean difference -0.65 (95% CI -1.88, 0.59).

Interestingly, another recently published study showed that oral sucrose does not significantly affect activity in neonatal brain or spinal cord nociceptive circuits, and therefore might not be an effective analgesic agent.²⁶ In this double-masked, randomised controlled trial, 59 newborn infants were assigned to receive 0.5 ml 24% sucrose solution or 0.5 ml sterile water 2 minutes before undergoing a clinically required heel lance. The primary outcome was pain-specific brain activity evoked by one time-locked heel lance, recorded with electroencephalography and identified by principal component analysis. Secondary measures were the PIPP score, and spinal nociceptive reflex withdrawal activity. The PIPP score was significantly lower in infants given sucrose than in those given sterile water and significantly more infants had no change in facial expression after sucrose administration (mean 5.8, 95% CI 3.7-7.8 vs 8.5, 7.3-9.8; p=0.02). However, the nociceptive brain activity after the noxious heel lance did not differ significantly between infants who received sucrose and those who received sterile water (sucrose: mean 0.10, 95% CI 0.04–0.16; sterile water: mean 0.08, 0.04-0.12; p=0.46). No significant difference was recorded between the sucrose and sterile water groups in the magnitude or latency of the spinal nociceptive reflex withdrawal recorded from the biceps femoris of the stimulated leg. The investigators in this study concluded that the ability of sucrose to reduce clinical observational pain scores after noxious events in newborn infants should not be interpreted as pain relief. Perhaps oral sucrose distracts the baby from the painful stimuli giving a lower PIPP score which is misinterpreted as pain relief.

Non-nutritive sucking

Non-nutritive sucking has been investigated as an option for pain relief during screening for ROP. Boyle et al.²¹ (Table 2) evaluated the use of oral sucrose and/or pacifier for reducing pain responses during eye examinations. The infants randomized to pacifiers scored lower than those without pacifiers and there was no difference between groups receiving sucrose and those receiving water .The authors concluded that non-nutritive sucking with a pacifier reduced distress responses in infants undergoing screening for ROP.

Developmental Care

Other non-pharmacological interventions have been investigated as a possible method of pain relief. The Newborn Individualized Developmental Care and Assessment Program (NIDCAP) is an intervention program aiming at optimizing and adapting neonatal care for preterm infants.²⁷⁻³⁰ It is believed that NIDCAP-based interventions ("developmental care" interventions) may exert positive influences on pain and stress responses during routine care procedures, for example, diaper changes or weighing.

Slevin et al.³¹ investigated the degree of distress caused by ROP screening in a cohort of preterm infants and the effects of nesting in reducing their discomfort. This nesting concept was derived from developmental recommendations about the nursing care of preterm infants.²⁹ Before screening, the nested infants were placed on a soft padded surface with boundaries that helped to maintain and support them in a flexed position but still allowed unrestricted movement of their body and limbs. The non-nested infants were placed on a standard cot blanket without any boundaries. Both groups of infants were thus able to move freely without any restriction. From this study, the investigators concluded that the distress caused by ROP screening was significantly less for the nested group compared with the non-nested group for both movement activity and crying (duration of crying for nested 11.8 sec, for non-nested 92.4 sec; p<0.01). The limitation of this study is that it did not use a standardized pain scoring tool.

In another study, Kleberg et al.³² evaluated the effect of NIDCAP on newborn stress during ROP assessment. NIDCAP is a whole educational program but for the purpose of this study, short-NIDCAP based interventions ("developmental care" interventions) were used. This involved developmental care strategies such as undisturbed periods, incubator covers, bed support, and reduction in environmental light and noise. In this study, the first two eye examinations in thirty-six preterm infants were evaluated. The infants were randomly assigned at the first eye examination to receive either NIDCAP or standard care. The investigators assessed PIPP scores and salivary cortisol at defined time points up to four hours after the eye examination. There was no difference in PIPP score between the two care strategies before or after the eye examination. Salivary cortisol increased from baseline to 30 minutes after the eye examination independent of care strategy and decreased significantly between 30 and 60 minutes when infants were subjected to NIDCAP but not after standard care. The investigators concluded NIDCAP-based intervention during eye examination does not decrease pain responses but results in faster recovery, as measured by lower salivary cortisol 60 minutes after the examination. The limitation of this study is that there is no control group in which no eye study is performed to assess the response of the baby to the study procedures independent of the eye examination itself.

Rush et al. investigated the benefit of combined comfort care and pacifier soaked with 24% sucrose solution (Table 2).²⁵ The investigators found that the vital signs did not vary significantly between the two groups. The participants in the control group had a trend towards a longer crying time, but this trend did not reach statistical significance. In addition, the time required for the vital signs to return to their baseline values did not vary significantly. The authors concluded the routine use of comfort care to reduce pain during the examination could not be supported by this study. The limitation of this study is that the authors used different methods of pain relief (swaddling, pacifier and sucrose 24%) which could have confounded the final results.

ROP screening technique

Do examination techniques for ROP screening of babies have an impact on pain? There is currently conflicting information on the severity of pain experienced by a baby undergoing different modes of ophthalmology examination. Mehta et al.³³ compared the physiological and behavioral changes in premature infants undergoing three different methods of screening for ROP. In this prospective randomized cross-over study, fifteen premature infants requiring screening for ROP were recruited, and physiological and behavioral responses produced by three different methods of screening were compared. The screening methods used a retinal camera with eyelid speculum and an indirect ophthalmoscope with and without an eyelid speculum. Physiological indices (change in pulse, mean blood pressure and oxygen saturation) and facial responses to pain (brow bulge, eye squeeze, nasolabial fold, mouth opening and the presence of cry) were recorded at five points: before, during and immediately after screening and 10 and 30 min after examination. The investigators found that screening with the retinal camera and the indirect ophthalmoscope with a speculum both caused a greater change in pulse and mean blood pressure and an increase in facial responses during and immediately after screening as compared to the indirect ophthalmoscope without the speculum.

Muherjee et al.³⁴ carried out a study on a larger cohort of preterm infants and compared the impact of ROP screening examination between a digital retinal camera and conventional binocular indirect ophthalmoscope (BIO) using cardiorespiratory indices as a measure of distress. The investigators did not use the PIPP score. Eighty-six preterm infants with a birth weight of ≤ 1500 g or gestational age of ≤ 32 weeks and undergoing ROP screening were included. Heart rate (HR), oxygen saturation, respiratory rate (RR), and mean blood pressure (BP) were recorded before, during, and 1 hour after examination. The increase in HR (mean 23.4 ± 28.6 vs 13.7 ± 25.2; p=0.038) and RR (mean 11.1±17.9 vs 1.3±15.3; p=0.01) was significantly higher in the indirect ophthalmoscope group than in the digital camera group. The investigators concluded that screening for ROP with a digital retinal camera was associated with a significantly lower stress-related response than with a conventional indirect ophthalmoscope.

In a recently published prospective, randomised comparative study, 76 premature infants with a mean GA of 28.6 weeks needing ROP examination were randomized to undergo either examination with BIO or with a wide field retinal camera.³⁵ A lid speculum and scleral indenter were routinely used for indirect ophthalmoscope examination. The digital retinal examination was carried out with a lid speculum but scleral indentation was not routinely performed. Infants were examined on a cot blanket. They were unswaddled and non-nested. Pacifiers were not used and oral sucrose was not given. The PIPP scoring system was used to determine the severity of pain experience by the patients. Baseline observations were recorded and during the first minute of the examination. The PIPP score was assessed by an independent observer, who could not be masked to the type of examination. From this study, the investigators found that PIPP score was elevated during both types of examination (mean PIPP score for retinal camera 15.0 ± 2.1 , BIO 15.2 ± 2.4 ; p=0.47) but there was no statistically significant difference between the two different types of examination. The conclusion from this study was that both types of examination with eyelid speculum are similarly painful for infants. The authors proposed that the eyelid speculum rather than the examination method contributed most to the pain experienced.

Effects of feeding of pain during ROP examination

The effect of feeding on pain during ROP examination was investigated by Strube et al.³⁶ This prospective, randomized, single-masked study involved infants in the neonatal intensive care unit who required an ROP eye examination and who received normal or full enteral feeding. Infants were randomly assigned to 1 of 2 study arms: feeding 1 hour before examination (arm 1) or feeding schedule adjusted to ensure no feeding within 2 hours before examination (arm 2). No formal pain scoring was used; instead blood pressure and pulse rate, before, during and after examination, crying time during the examination was recorded. The presence of vomiting and gastric aspirates volume 24 hours after the examination were recorded. The investigators found that there was 19% less crying (p = 0.016) in arm 1 versus arm 2. Vomiting or gastric aspirates were the same between both groups. They concluded that feeding neonatal intensive care unit infants 1 hour before compared with withholding feeding 2 or more hours before ROP examinations may reduce stress during the examination.

A Cochrane review on effect of breast feeding or breast milk for procedural pain in neonates concluded that breast milk should be used to alleviate pain in neonates undergoing a single painful procedure compared to placebo.³⁷ Breast milk was found to be as effective as sucrose. However, all 11 studies reviewed in the systematic review were of babies undergoing venepuncture or heel lancing for neonatal screening and none were from babies undergoing ROP eye examination.

Conclusion

Ophthalmological examination is a painful procedure. However, it is a very essential part in the management of premature infants. A topical anesthetic agent marginally decreases pain and could be used during examination. Speculum insertion appears to be the most uncomfortable aspect of the examination. Non-pharmacological intervention such as non-nutritive sucking with a pacifier and nesting may be beneficial. There is no role for routine use of oral sucrose and there is evidence to suggest that it might not have any effect on pain perception. Currently available data suggest that babies should be fed as usual during eye examination and delaying feeds has no recognized benefit. Breast milk is a possible alternative for pain relief but more studies are needed to establish its benefit during ROP examination.

Literature search

A literature search was conducted by the authors using MEDLINE (January 1980 to January 2011) and Cochrane Central Register of Controlled Trials Issue 1 of 4, January 2011, using the following keywords: retinopathy of prematurity, screening examination, neonates, pain relief and stress. Only articles published in English were considered.

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Study	Method	Participants	Intervention	Outcome	Results
Saunders et al. ¹⁶	Randomized double masked control trial	42 infants, GA from 35 to 45 weeks (corrected age)	Proparacaine HCL 0.5% (cases) and normal saline (control)	No formal pain scoring – vital signs and cry recorded*	Analysis of variance used to compare parameter at 0, 2 and 5 minutes. <i>P</i> value >0.05 for all comparisons
Marsh et al. ¹⁷	Randomized double masked cross-over control trial	22 infants, GA from 24 to 32 weeks	Proparacaine HCL 0.5% (cases) and normal saline (control)	PIPP (baseline score measured prior to examination and then 0, 1 and 5 minutes during examination	PIPP score at speculum insertion (0 minute) is lower in treatment group (paired difference -2.5 ± 3.4 ; p=0.001), 1 minute, paired difference -1.2 ± 4.0 , P=0.09, 5 minutes, paired difference -1.3 ± 3.6 , P=0.06
Mehta et al. ¹⁸	Randomized double masked cross-over trial	50 infants, GA 26 to 31 weeks (assessment done at GA 33.3 and 35.3 weeks)	Proparacaine HCL 0.5% (cases) and normal saline (control)	PIPP (baseline score measured 1 minute prior and then 1 and 5 minutes during examination.	For 1 st examination, PIPP score at 1 minute 11.725 (saline) vs. 10.375 (Proparacaine) (P=0.013) PIPP score at 5 minutes 5.225 (saline) vs. 6.550 (Proparacaine) (P=0.767)
Cogen et al. ¹⁹	Randomized double masked control trial	34 infants, GA 31 to 40 weeks	Proparacaine 0.5 % (cases) and artificial tears (control)	PIPP (scores measured at speculum insertion, indirect ophthalmoscopy with and without sclera depression)	PIPP score \geq 11 during 65% of the examinations without topical anesthesia compared with 27% examination with anesthesia (P=0.04)

Table 1. Studies comparing the use topical of anesthesia in infants undergoing eye examination for ROP

*Heart rate, respiration rate, BP, and transcutaneous oxygen saturation, infant cry and corneal opacity. GA (gestational age),PIPP (Premature Infant Pain Profile)

Study	Method	Participants	Intervention	Outcomes	Results
Boyle et al. ²¹	Randomized controlled trial	40 preterm infants with GA 24-34 weeks (median 29 weeks)	 Sucrose 33% and sterile water ± pacifier (randomized to four interventions): 1) 1 ml sterile water 2) 1ml 33% sucrose solution 3) 1 ml sterile water with pacifier 4) 1 ml 33% sucrose solution with pacifier 	PIPP (during examination of first eye)	Mean PIPP scores were 15.3, 14.3, 12.3, and 12.1 for groups 1, 2, 3, and 4 respectively. ANOVA showed a significant difference score between groups (p =0.023). Infants randomized to pacifiers scored lower than those without pacifiers (t test for equality of mean 95% CI -4.23, -0.96; p =0.003). No significant difference between groups receiving sucrose and those receiving water (t test for equality of mean 95% CI -0.92, 2.74; p=0.321).
Gal et al. ²²	Randomized double- masked controlled trial	23 preterm infants with GA 24-29 weeks	2m1 Sucrose 24% and 2 ml sterile water (both groups also received proparacaine HCl)	PIPP (baseline scores 1 and 5 minutes pre-exam, at initial placement of the eye speculum and 1 and 5 minutes after examination)	Score during speculum insertion lower in sucrose group (paired difference -2.2 ± 3.9 , $p=0.01$) Effects not sustained at 1 and 5 minutes, paired difference -0.6 ± 4.1 , $p=0.24$; paired difference -0.9 ± 2.7 , $p=0.07$ respectively

 Table 2. Studies using oral sucrose for pain relief in infants undergoing eye examination for ROP

Grabska et al. ²³	Randomized masked control trial	32 preterm infants mean GA 28 weeks	Sucrose 24 % and sterile water (both groups received the topical tetracaine, swaddled and given pacifier; sucrose adjusted to body weight)	PIPP (Baseline measurement, during examination and at 1 minute intervals post examination)	PIPP score during examination 14±3 for both group (no significant difference)
Mitchell et al. ²⁴	Randomized double- masked controlled trial	30 preterm infants with mean GA 26.5 for treatment group and 27.3 weeks for control	Sucrose 24% 0.1 ml (3 doses) and sterile water 0.1 (3 times) (both groups were given pacifier and received proparacaine HCl)	PIPP (Baseline measurement, during examination and at 30, 60, 90 and 120 seconds post examination)	PIPP scores during examination for the sucrose group was significantly lower than in control group 8.8 ± 0.7 and 11.4 ± 0.6 respectively, p=0.0077
Rush et al. ²⁵	Randomized control trial	30 preterm infants with mean GA 29.57 for treatment group and 28.8 for control	Pacifier soaked with Sucrose 24% and swaddling for treatment group. Study group received none of the above (both groups received proparacaine)	None (Pulse rate, respiratory rate and oxygen saturation monitored 30 and 5 minutes before, during and 5 minutes after the examination)	No significant difference in the measured parameters between both groups (p >0.05)

GA (gestational age), PIPP (Premature Infant Pain Profile)

Original research articles

- Kandasamy Y, Smith R, Wright IMR, Hartley L. Relationship between birth weight and retinal microvasculature in newborn infants. *Journal of Perinatology*. 2012; 32(6):443-7.
- Kandasamy Y, Smith R, Wright IMR. Retinal microvasculature measurements in fullterm newborn infants. *Microvascular Research*. 2011; 82(3):381-4.
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- Kandasamy Y, Smith R, Wright IMR, Lumbers ER. Relationships between glomerular filtration rate (GFR) and kidney volume in low-birth-weight neonates. *Journal of Nephrology*. 2012; Oct 3.
- Kandasamy Y, Smith R, Wright IMR, Lumbers ER. Extra-uterine renal growth in preterm infants. (undergoing peer review)

Relationship between birth weight and retinal microvasculature in newborn infants

Yogavijayan Kandasamy, Roger Smith, Ian MR Wright and Leo Hartley Journal of Perinatology. 2012; 32(6):443-7

Abstract

Objective: The purposes of this study were to determine the normal retinal microvasculature measurements in human infants who are born at term and to determine whether birth weight influences measurements of retinal microvasculature.

Study Design: Retinal arteriole and venule measurements were obtained in a cohort of 24 infants who were born at term. Digital images of both retinas were obtained using a digital retinal camera after pupillary dilation.

Result: Twenty-four newborn infants born at term (12 females and 12 males) were analyzed in this study. The measured retinal arteriole diameters were $66.8-147.8 \mu m$ (mean, $94.2 \pm 19.6 \mu m$), and the venule diameters were $102.0-167.8 \mu m$ (mean, $135.2 \pm 19.1 \mu m$). Seven babies in the sample had low birth weight, while 17 babies were born with normal weight. Babies with lower birth weights had larger arteriole ($113.1 \pm 17.9 \mu m$ vs. $86.4 \pm 14.4 \mu m$; P = 0.0009) and venule diameters ($151.7 \pm 14.9 \mu m$ vs. $128.4 \pm 16.9 \mu m$; P = 0.0040).

Conclusions: Retinal venules and arterioles in low-birth-weight babies are larger compared to those of normal-birth-weight babies. We postulate that the difference observed in our study was due to *in utero* pathophysiological changes that occurred in the cerebral circulation of growth-restricted fetuses.

Keywords: Retinal arterioles, retinal venules, middle cerebral artery, intrauterine growth restriction

Introduction

In utero insults that result in low-birth-weight (LBW) infants (birth weight < 2500 g)¹ are now well recognized as risk factors contributing to the development of vascularrelated diseases in adulthood.^{2, 3, 4, 5, 6} LBW infants are a heterogeneous group of infants, comprising infants who are premature (< 37 completed weeks of gestation),¹ growthrestricted (weight below the 10th percentile for their gestational age),¹ or a combination of both. The exact mechanism of this phenomenon has yet to be fully understood, but there is increasing evidence to suggest that microcirculatory pathology forms the mechanistic link between fetal insult and the adult manifestation of illness.^{7, 8, 9, 10, 11} The challenge has been to investigate microcirculatory changes in vivo. The retina provides an opportunity for in vivo investigation of human microcirculation, and changes in the retinal vessels have been identified in some individuals who had LBW as infants and later developed hypertension, ischemic heart disease, stroke, and renal disease.^{9, 12, 13, 14,} ¹⁵ The ability of retinal-imaging technology to assess and measure the retinal microvasculature makes this a very valuable assessment tool.^{16, 17, 18} Studies involving young children, adolescents, and adults who were born small have shown abnormalities in the retinal vasculature.^{9, 12, 15, 19, 20, 21, 22, 23}

Although the retinal microvascular of premature infants is routinely assessed to detect and treat Retinopathy of prematurity (ROP),²⁴ there are no published studies regarding the use of retinal-imaging technology to assess the retinal microvasculature of at-term, growth-restricted infants. There are also no published data concerning normal measurements of retinal microvasculature in infants. The purpose of the present study was to determine normal measurements of retinal microvasculature in human infants who are born at term. This study also investigated whether birth weight influences measurements of retinal microvasculature.

Materials and Methods

This study was performed in the Department of Neonatology, The Townsville Hospital, Queensland, Australia. The Department of Neonatology is a tertiary perinatal center catering to more than 10 000 births each year. The study commenced in August 2010, and the data presented in this study are based on patients recruited until May 2011. This study was approved by the Townsville Health District Human Research Ethics Committee. Written parental consent was obtained, and babies with syndromes, prematurity, and chromosomal abnormalities were excluded. All assessments were performed within the first 7 days of life. Babies with birth weights of < 2500 g were classified as LBW babies, and babies weighing 2501–4500 g were classified as Appropriate for gestational age (AGA) babies. Only babies who were born at term (37 weeks of gestation completed) were included in this study.

After pupillary dilation, digital images of both retinas were obtained using a digital retinal camera (RetCam, Massie Laboratories, Dublin, CA, USA). Measurements of the diameters of retinal vessels were then obtained using a predetermined protocol that first involved the identification of retinal vessels located 0.5–1 disc diameter from the margin of the optic disc (Figure 1). Measurements of vessel diameter were then obtained using semi-automated software (Vesselmap, IMEDOS GmbH, Jena, Germany).^{25, 26} Vascular diameter was computed as wall-to-wall distance within the vessel. The caliber of directly viewed vessels was determined by the size of the red-cell column, because the vessel walls and peripheral plasma layer are nearly transparent.²⁷ Measurements of vessels from each eye were obtained, and the largest venule and arteriole for each patient was determined. These measurements were then used for analysis. An intra-class correlation coefficient was 0.90 (95% confidence interval of 0.75-0.96). A previously published study in infants has shown that the blood flow in the central retinal arteries is similar in both the eyes.²⁹

In adult eyes, correction can be applied to compensate for inaccuracies in the measurements of retinal structure that occur because of refractive error; this correction requires parameters such as axial length and keratometry (curvature of the anterior surface of the cornea) to be known.³⁰ In infants, these calculations are more challenging because obtaining these measurements is difficult and the eye is continuing to grow.³⁰ Statistical analysis was carried out using Stata ver. 11.0 (Stata Corp, College Station, TX, USA). Using Student's *t*-test, P values < 0.05 were considered significant.

Results

A total of 247 babies were admitted to the department during the study period. Of these, 99 were suitable for recruitment, and their parents were approached for participation. Written consent was obtained for 24. All 24 newborn infants born at term (12 females and 12 males) were analyzed in this study. Birth weights ranged from 1845 to 4310 g (mean, 3029 ± 649 g) with gestational ages of 37–41.6 weeks (mean, 38.7 ± 1.4 weeks). Retinal arteriole diameters were $66.8-147.8 \mu m$ (mean, $94.2 \pm 19.6 \mu m$), and venule diameters were $102.0-167.8 \mu m$ (mean, $135.2 \pm 19.1 \mu m$). Table 1 compares the differences in these measurements between male and female infants.

The infants were divided into 2 cohorts based on birth weight (LBW and AGA). There were 7 LBW babies and 17 AGA babies. Babies with LBW had larger arteriole (113.1 \pm 17.9 µm vs. 86.4 \pm 14.4 µm; P = 0.0009) and venule diameters (151.7 \pm 14.9 µm vs. 128.4 \pm 16.9 µm; P = 0.0040). Figure 2 shows the relationship between birth weight and vessel diameters. Pearson's coefficient of correlation between retinal arteriole and venule diameter was 0.7522 (95% CI 0.50 – 0.89; P < 0.0001) (Figure 3).

Discussion

To date, studies of retinal vasculature in infants have mainly focused on premature infants and ROP.^{25, 26} To our knowledge, this is the first study to investigate measurements of retinal microvasculature using digital retinal imaging in infants born at term. These measurements could be used as a baseline for future studies that investigate the effects of birth weight on retinal microvasculature. Previous studies have shown a strong relationship between LBW and retinal vasculature size in older children,^{15, 19, 22, 23, 31} adolescents,³² and adults.^{9, 12, 13} However, no published studies utilized baseline measurements of infant retinal vasculature for comparison. For the first time, we were able to measure retinal arteriole and venule sizes in LBW infants during infancy. The data from this study show significantly higher retinal vessel diameters in LBW babies. By contrast, previously published studies of young children^{15, 22} have shown that children who were born as LBW infants had narrower retinal arteriolar calibers. Narrowing of these vessels has been linked to the development of cardiovascular diseases in adults.^{7, 14, 33}

Why are the diameters of retinal vessels significantly larger in LBW babies? Only infants born at term were reviewed in this study; thus, the cause of LBW in this cohort was intrauterine growth restriction (IUGR). The retinal images in this study were all taken during the first week of life, so we propose that the differences observed in our study were due to pathophysiological changes that occurred *in utero*. There are many

causes of IUGR, but the most common is uteroplacental insufficiency, which results in fetal hypoxia.^{34, 35}

Fetal cerebrovascular responses to hypoxia are fundamentally different from those observed in the cerebral circulation of adults.³⁶ The vasculature of the immature brain is highly plastic and can respond to hypoxia with robust increases in capillary density.³⁶ Endothelial vasodilator capacity is typically depressed in fetal cerebral arteries, and the endothelium contributes relatively little to hypoxic vasodilatation in the fetus.^{36, 37} By contrast, the endothelial contribution to hypoxic vasodilatation increases throughout early postnatal life, becoming quite prominent in the cerebral arteries of adults.^{37, 38} Hypoxia exerts effects on vascular smooth muscle through various mechanisms.³⁶ The smaller and more peripheral cerebral arteries relax quickly and completely in response to hypoxia, whereas the larger and more proximal arteries, including the common carotid, maintain muscle tone much better and play a more important role in the gradual adjustments of cerebrovascular tissue to resist hypoxia.³⁹

Animal studies have provided insight into some aspects of the basic pathophysiology of IUGR, and studies using technologies such as Doppler ultrasound to investigate maternal and fetal vessels have added further information. Doppler ultrasound allows for the assessment of the vascular effects of placental dysfunction on the placental and fetal vasculature.⁴⁰ In response to hypoxia, the fetus uses a compensatory mechanism to redistribute cardiac output and blood supply to the brain to maintain constant blood delivery to this organ (the head-sparing effect).⁴¹ The result is a decrease in cerebral blood-flow resistance and vasodilatation of the arteries. This effect, which can be measured using Doppler ultrasound, shows a decrease in resistance and an increase in blood flow in the middle cerebral artery.⁴⁰ Studies of growth-restricted fetuses have confirmed dilatation of the middle cerebral artery and the resulting increase in blood flow to the brain compared to fetuses with normal growth.^{42, 43, 44}

Figure 4 shows the close relationship between the retinal artery and the middle cerebral artery. The endothelia of the vessels in the brain and retina are lined with continuous endothelial cells, connected by tight junctions that help to maintain the blood–brain barrier.⁴⁵ Doppler flowmetry data from newborn babies have shown that an increase in blood flow in the middle cerebral and ophthalmic artery is closely followed by an increase in blood flow in the central retinal artery.²⁹ Blood flow in the middle cerebral artery and temporally coupled to fetal

brain function and metabolism, and we postulate that, in a growth-restricted fetus, the same neurovascular coupling extends to the retinal artery. Dilation of the middle cerebral artery possibly results in dilatation of retinal vessels in growth-restricted, LBW infants.

The main limitation of our study was its relatively small sample size. It was also difficult to account for any refractive error that could have contributed to the results. We plan to follow this cohort over time to identify the changes in retinal vasculature as these infants grow.

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	Male	Female	P value
Number	12	12	
Birth weight (gm)	3017 ± 537	3041 ± 770	0.9282
Gestation (weeks)	38.3 ± 1.1	39.2 ± 1.4	0.0994
Venule diameter (µm)	130.2 ± 18.8	140.3 ± 18.9	0.2029
Arteriole diameter (µm)	90.1 ± 17.2	98.2 ± 21.6	0.3206

Table 1. Comparison of measurements between male and female infants



Figure 1. Retinal image from a newborn infant showing identification and measurement of retinal vessels 0.5 to 1.0 disc diameters from the margin of the optic disc.



Figure 2. Relationships between birth weight and the diameters of retinal arterioles and venules in infants born at term.



Figure 3. Increasing retinal arteriole diameter is closely correlated with increasing retinal venule diameter.



Figure 4. Diagram showing the Circle of Willis, middle cerebral artery, and origin of the ophthalmic and central retinal arteries.

Retinal microvasculature measurements in full-term newborn infants

Yogavijayan Kandasamy, Roger Smith, Ian MR Wright Microvascular Research. 2011; 82(3):381-4

Abstract

Objective: Currently, there are no published data on retinal microvasculature size in human infants born at term. The purpose of this study was to determine the normal retinal microvasculature measurements in human infants born at term with normal birth weight and to compare these results with measurements in children and adults. **Methods:** Retinal arteriole and venule measurements were obtained in a cohort of 20 full-term infants. Digital retinal images were obtained from both eyes after pupillary dilation using a digital retinal camera. Measurements of vessel diameter were then obtained using semi-automated software.

Results: Twenty infants (9 female infants and 11 male infants) were analyzed. The retinal arteriole diameter was $66.8-123.0 \mu m$ (mean, $85.5 (14.3) \mu m$), and the venule diameter was $102.0-167.8 \mu m$ (mean, $130.0 (16.0) \mu m$). There were no differences in the arterial or venule diameters between the male and female infants ($83.2 (12.2) \nu s$. 88.3 (16.9); P = 0.4372; $124.3 (16.0) \nu s$. 137.0 (18.0); P = 0.08). The arteriovenous ratio was found to be 0.66 (95% CI 0.62-0.71). The coefficient of correlation between the retinal arterioles and venules was 0.56. The retinal arteriole and venule diameters increase as a person matures. The arteriovenous ratio also increases with age.

Conclusion: In newborn infants, retinal venules are significantly larger than retinal arterioles. The arteriovenous ratio is smaller in neonates compared to adults indicating the retinal arteriole diameter increases at a different pace compared to retinal venule. Sex does not influence the retinal microvasculature size in infants. The presence of spontaneous retinal hemorrhage and the inability to account for refractive errors were the main limitations of this study.

Keywords: retinal arteriole, retinal venule, low birth weight, middle cerebral artery, intrauterine growth restriction

Introduction

Low-birth-weight (LBW) (birth weight < 2,500 g) is now well-recognized as a risk factor for the development of vascular-related diseases in later life (Barker, 2004; Barker, 2006a; Barker, 2006b; Barker et al., 2005; Barker et al., 1989). LBW infants are a heterogeneous group, comprising newborns who are premature (< 37 completed weeks of gestation), growth-restricted (weight below the 10th percentile for their gestational age), or a combination of both. The exact mechanism of this phenomenon remains to be fully understood, but there is increasing evidence to suggest that microcirculatory pathologies form the mechanistic link between in utero insult and the adult manifestation of illness (Liew et al., 2008a; Martin et al., 2000; Mimoun et al., 2009; Sasongko et al., 2010; Struijker-Boudier et al., 2007). The challenge has been to investigate microcirculatory changes in vivo. The human retina provides a unique opportunity for in vivo investigation of microcirculation, and changes in the retinal vessels have been identified in some individuals who had LBW as infants and later developed cardiovascular and/or renal disease (Chapman et al., 1997; Hellstrom et al., 2004; Liew et al., 2008a; McGeechan et al., 2009; Sun et al., 2009). The ability of retinal imaging technology to assess and measure the retinal microvasculature makes this an important assessment tool (Chapman et al., 2001; Liew et al., 2008b; Wong et al., 2004a).

The retinal microvasculature of premature infants is routinely assessed to detect and treat retinopathy of prematurity (ROP) (Section on Ophthalmology et al., 2006). However, there have been no published studies regarding the use of retinal imaging technology to assess the retinal microvasculature of well full-term infants. There are also no published data concerning normal baseline measurements of retinal microvasculature size in full-term infants. The objective of this study was to determine measurements of retinal microvasculature in human infants born at term with normal birth weight (NBW) (weighing 2,500-4,499 g). We then compared these measurements with those from children and adults.

Materials and Methods

This study was performed in the Department of Neonatology, The Townsville Hospital, Queensland, Australia. The Department of Neonatology is a tertiary perinatal center responsible for more than 10,000 births each year. The study commenced in August 2010, and the data presented in this report are based on patients recruited until June 2011. This study was approved by the Townsville Health District Human Research Ethics Committee, was conducted in compliance with good clinical practice guidelines, institutional review board regulations, and written consent from parents, and was in accordance with the tenets of the Declaration of Helsinki. Babies who needed respiratory support, surgery, infants of gestational diabetes mothers, were excluded, as were those with syndromes, prematurity, LBW or chromosomal abnormalities. All assessments were performed within the first 7 days of life. Only babies who were born at term (37 weeks of gestation completed) with NBW were included in this study. After pupillary dilation, digital images of both retinas were obtained using a digital retinal camera (RetCam, Massie Laboratories, Dublin, CA, USA). Measurements of the diameters of the retinal vessels were then obtained using a predetermined protocol that first involved the identification of retinal vessels located 0.5-1 disc diameter from the margin of the optic disc (Figure 1). We provided pain relief with oral sucrose and local anaesthetic agents for the eye examination. Pupillary dilation was carried out using cyclopentoate and phenyephrine ophthalmic drops. The procedure was carried out by two persons – a neonatologist and a neonatal nurse. The most reliable images were obtained when the infant remained calm and had adequate papillary dilatation. The focus button on the control panel was used to obtain the clearest image. Still retinal images with optic disc in the centre were taken from both retinas. The sharpest image was then chosen for vessel measurement.

Measurements of vessel diameter were then obtained using semi-automated software (Vesselmap, IMEDOS GmbH, Jena, Germany) (Grunwald et al., 2009; Johnson et al., 2007). The vessel diameter was computed as the distance between the walls within the vessel. The caliber of directly viewed vessels was determined by the size of the red cell column, because the vessel walls and peripheral plasma layer are nearly transparent (Archer et al., 2010). Vessels were measured in each eye, and the largest venule and arteriole of each patient was determined. These measurements were then analyzed. An intraclass correlation coefficient was used to determine the reliability of this technique (Bland and Altman, 1986): this correlation coefficient was 0.90 (95% CI 0.75-0.96). A previously published study in infants has shown that the blood flow in the central retinal arteries are similar in both eyes (Papacci et al., 1998a).
Statistical analyses were performed using MedCalc Version 11.6 (MedCalc Software bvba, Mariakerke, Belgium). Using Student's t-test, P values < 0.05 were considered significant. The normality of variables was determined using the D'Agostino-Pearson test (D'Agostino et al., 1990).

Results

A total of 254 babies were admitted to the department during the study period. Of these, 106 were suitable for recruitment, and their parents were approached for participation. Written consent was obtained for 31 infants. Ten babies were excluded because of LBW. Three infants were found to have spontaneous retinal hemorrhage, and 1 of these was excluded because of bilateral and diffuse retinal hemorrhage (Figure 2). Twenty newborn infants born at term (9 female infants and 11 male infants) were analyzed in this study. Their birth weights ranged from 2,500 to 4,310 g (mean, 3,342 (523) g), and their gestational ages ranged from 37 to 41.6 weeks (mean, 39.2 (1.4) weeks). The retinal arteriole diameters were 66.8–123.0 µm (mean, 85.5 (14.3) µm), and the venule diameters were 102.0–167.8 µm (mean, 130.0 (16.0) µm). Table 1 compares the retinal arteriole and venule measurements in the term infants. The size of the retinal arterioles was significantly smaller than that of the retinal venules (85.5 vs. 130.0 μ m; P < 0.00001). The mean AVR was 0.66 (95% CI 0.62–0.70). The coefficient of correlation between the retinal arteries and veins was 0.56 (Figure 3). There were no differences between male and female infants, as shown in Table 2. These results were then compared to published retinal microvasculature measurements from children and adults (Table 3).

Discussion and Conclusions

Studies of retinal vasculature in infants have mainly focused on premature infants and ROP (Grunwald et al., 2009; Johnson et al., 2007). To our knowledge, this is the first study to investigate measurements of retinal microvasculature using digital retinal imaging in NBW term infants. These measurements could be used as a baseline for future studies that investigate the effects of birth weight and other neonatal conditions on retinal microvasculature. Previous studies have shown a strong correlation between LBW and retinal vasculature size in older children (Cheung et al., 2008; Hellstrom et al., 1997; Mitchell et al., 2008; Sun et al., 2009; Tapp et al., 2007), adolescents (Gopinath et

al., 2010), and adults (Chapman et al., 1997; Hellstrom et al., 2004; Liew et al., 2008a). However, no published studies have utilized the baseline measurements of infant retinal vasculature for comparison.

Compared to previously published results, retinal arteriole and venule diameters nearly double by the time a child is 6 years old (Mitchell et al., 2008). Over the same period of time, the body weight of the infant would have increased more than fivefold (WHO, 2006). In young children and healthy adults, the retinal arteriole remains smaller than the retinal venule (Hughes et al., 2009; Mitchell et al., 2008; Wong et al., 2004a). The AVR is an important measure of retinal microvasculature that combines information from both arterial and venous system and has the advantage of controlling for magnification differences between camera lenses and refractive errors (Liew et al., 2007). Our study demonstrated the AVR in an NBW infant to be 0.66 and this ratio increased in adults to 0.89 (Wong et al., 2004a). The available data suggest that while retinal venule diameter remains approximately the same from the age of 6 years onwards, the retinal arteriole diameter continues to grow from infancy to adulthood resulting in an increased AVR in a healthy adult (Mitchell et al., 2008; Wong et al., 2004a). The significance of this finding is that deviation of AVR from a normal value, in particular a lower AVR, has been associated with increased risk of stroke (Wong et al., 2001), hypertension (Wong et al., 2004b) and cardiovascular disease (Wang et al., 2006).

One of the main challenges that we faced in the newborn term infants in our study was spontaneous retinal hemorrhage. Three of the 21 patients (14%) in our cohort were found to have spontaneous retinal bleeding, and one had to be excluded because of diffuse bilateral bleeding, as shown in Figure 2. The hemorrhage rendered measurements difficult and inaccurate. Spontaneous retinal hemorrhage is a well-recognized event in healthy newborns. The percentage of full-term infants who develop spontaneous retinal hemorrhage at birth ranges from 10 to 30% (Hughes et al., 2006; Kaur and Taylor, 1990). The cause of this phenomenon is unknown. It does not require any treatment and resolves spontaneously. This condition is not related to the mode of childbirth (Hughes et al., 2006).

Another limitation of our study is our inability to account for any refractive errors that could have influenced the measurements (Cheung et al., 2008). In adults' and children's eyes, corrections can be applied to compensate for inaccuracies in the

measurements of retinal structure that occur because of refractive error; these corrections require ocular biometric measurements, including axial length, anterior chamber depth, lens thickness, vitreous chamber depth parameters, and keratometry (measurements of the curvature of the anterior surface of the cornea) (De Silva et al., 2006). These measurements can easily be acquired in adults and young children. However, these calculations are more challenging in infants due to the continued growth of the eye and the inability of infants to remain still while these measurements are performed (De Silva et al., 2006).

The sample size in this study was limited, but we believe that the data obtained could be used as a baseline for future studies that investigate the role of retinal microvascular abnormalities in the development of cardiovascular diseases. We hope to perform a similar analysis in a cohort of LBW full-term infants in the future. Results shown in Table 3 were all obtained from different retinal cameras. Ideally, comparisons of retinal microvasculature between various age groups should be carried out using images produced by a similar retinal camera. However, the currently available retinal camera for use with neonates is not suitable for children and adults. Perhaps in the future, when such technology is available, the comparisons will be more accurate.

In conclusion, the retinal microvasculature in human increases in diameter as an infant grows. The retinal venule stops growing in childhood whereas the retinal arteriole continues to grow until adulthood. The net result is an increase in AVR. Deviation from a normal AVR, particularly a low AVR, has been associated with an increased risk of vascular diseases.

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Variable	Venule diameter	Arteriole diameter	
Number of infants	20	20	
Diameter (µm)	102.0-167.8	66.8-123.0	
Mean (µm)	130.0 ± 16.0	85.5±14.3	
95% CI for the mean	122.5 to 137.5	78.8 to 92.2	
Median (µm)	130.2	86.2	
95% CI for the median	124.5 to 138.0	75.0 to 92.9	
D'Agostino-Pearson test	Normal distribution (P=0.6289)	Normal distribution (P=0.1918)	

 Table 1. Summary statistics comparing retinal arterioles and venules in normal-weight infants

Table 2. Comparison of measurements between male and female infants

Variables	Female	Male	P value	
Number	9	11		
Birth weight (g)	3478(508)	3232(532)	0.308	
Length (cm)	50.4(1.9)	49.4(3.0)	0.413	
Head circumference (cm)	34.4(0.7)	34.7(1.7)	0.634	
Arteriole diameter (µm)	88.3(16.9)	83.2(12.2)	0.437	
Venule diameter (µm)	137.0(18.0)	124.3(12.2)	0.078	
Arteriovenous ratio	0.64(0.10)	0.67(0.1)	0.591	

Table 3. Retinal microvasculature measurements in infants, children and adults

Age	Mean retinal arteriole diameter (µm)	Mean retinal venule diameter (µm)	Arteriovenous ratio
Newborn term infant	85.5	130.0	0.66
Children (6 years)*	165.6	232.0	NA
Adults (43-84 years)**	202.3	227.2	0.89

*Mitchell et al., 2008, **Wong et al., 2004, NA – not available



Figure 1. Retinal image from a newborn infant showing the identification and measurement of retinal vessels 0.5 to 1.0 disc diameters from the margin of the optic disc.



Figure 2. Spontaneous retinal hemorrhage in a full-term infant



Figure 3. Graph showing the correlation between retinal arteriole and venule diameters in normal-weight, full-term infants. Increase in retinal arteriole diameter is associated with an increase in retinal venule diameter.

Relationship between the retinal microvasculature and renal volume in low-birthweight babies

Yogavijayan Kandasamy, Roger Smith, Ian MR Wright

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Abstract

Objective: We carried out a study to assess whether the development of the retinal microvasculature reflects nephron growth and therefore nephron number.

Study design: In our study, we determined the association between kidney volume (nephron number) and the retinal microvasculature of term LBW and normal birth weight (NBW) infants (12 LBW and 26 NBW).

Results: LBW infants had significantly larger retinal arteriolar and venular diameters ($104.2 \pm 21.4 \text{ vs. } 87.0 \pm 12.7 \mu\text{m}$; p = 0.004; $146.8 \pm 19.5 \text{ vs. } 128.0 \pm 19.5 \mu\text{m}$; p = 0.01, respectively) compared with NBW infants. LBW infants also had smaller mean renal volumes ($9.3 \pm 2.3 \text{ vs. } 12.2 \pm 3.1 \text{ ml}$; p = 0.008). There were negative correlations between retinal arteriolar and venular diameters and renal volumes (r = -0.34, p < 0.05; r = -0.37, p < 0.05, respectively).

Conclusion: The larger the kidney (and, by implication, the greater the nephron number), the smaller are the diameters of retinal arterioles and venules. Thus, the degree of dilation of the retinal microvasculature provides an indirect index of renal growth.

Keywords: retinal vessels, renal volume, neonate, low birth weight

Introduction

Low birth weight (LBW; <2500 g)¹ constitutes a risk factor for adult renal disease^{2–5}. Globally, more than 20 million LBW infants are born each year⁶. Birth weight is strongly correlated with total glomerular number and glomerular size in the postnatal kidney. Total glomerular number has a direct linear relationship with birth weight, whereas mean glomerular volume is inversely correlated with glomerular number⁷. LBW infants constitute a heterogeneous group of babies who may be premature, growth restricted (birth weight below the 10th centile), or both. The critical window of the final stages of kidney development spans from 32 to 35 weeks of gestational age, and no new nephrons are formed after 36 weeks. Nephron endowment is, at that point, fixed for life^{3,8}.

The retina provides a unique opportunity for the *in vivo* investigation of the human microcirculation. The eye, kidney, and blood vessels are distinct organs that share common features in many systemic diseases⁹. In fact, the eye has been described as a window to the kidneys¹⁰. Histopathological changes that occur in both retinal and renal microcirculation in many clinical conditions are well recognized^{9,10}. Studies have shown that retinal microvascular diameters are predictive of renal function^{11–13}. In patients that have chronic kidney disease, the ratio of retinal arteriole to the venule lumen diameter has been found to be lower than that of matched controls¹⁴. The appearance of the retinal microvasculature is thus believed to be indicative of systemic microvasculature status, including the microvasculature of the kidneys.

Studies that have shown a link between LBW and reduced nephron endowment have mainly been based on autopsy findings^{7,15,16}. Furthermore, the results linking changes in the retinal microvasculature with renal function are predominantly from adult subjects^{11–13}. Therefore, we performed an *in vivo* study on LBW infants to determine whether kidney volume is associated with changes in the retinal microvasculature.

Methods

Participants

This cross-sectional study was performed in the Department of Neonatology, The Townsville Hospital, Queensland, Australia, a tertiary perinatal center responsible for more than 10,000 births each year. The study commenced in August 2010, and the data

presented in this report are based on patients recruited over a 12-month period. This study was approved by the Townsville Health District Human Research Ethics Committee, was conducted in compliance with good clinical practice guidelines, institutional review board regulations, and written consent from parents, and was in accordance with the tenets of the Declaration of Helsinki. Only babies who were born at term (i.e. after 37 weeks of gestation) were included in the study. Based on birth weight, infants were classified as term LBW or normal birth weight (NBW) (2500–4500 g). Infants who needed oxygen and/or respiratory support or surgery, those that were born with a large birth weight (>4500 g), and those born to mothers with gestational diabetes were excluded, as were those with syndromes, prematurity, or chromosomal abnormalities. All assessments were performed within the first seven days of life.

Retinal microvasculature measurements

Digital images of both retinas were obtained using a retinal camera (RetCam, Massie Laboratories, Dublin, CA, USA). This device is a contact retinal camera and the measurements could be affected by the cornea pressure. Hence, the measurements were only carried out by a trained operator (YK) and only minimal pressure was applied during assessment. The measurements of retinal vessel diameters were then obtained using a predetermined protocol that first involved the identification of retinal vessels located 0.5–1 disc diameters from the margin of the optic disc. We provided pain relief using oral sucrose and local anesthetic agents during the eye examination¹⁷. Pupillary dilation was carried out using cyclopentolate and phenylephrine ophthalmic drops. Video and still retinal images with the optic disc in the center were obtained and the sharpest image was then chosen to measure the vessels. Papacci et al. showed that the blood flow in the central retinal artery of infants is similar in both eyes¹⁸. Vessel diameter, computed as the distance between the walls within the vessel, was measured using semi-automated software (Vesselmap, IMEDOS GmbH, Jena, Germany)¹⁹⁻²¹. Thus, the caliber of directly viewed vessels was determined by the size of the red cell column, since the vessel walls and peripheral plasma layer were almost transparent²². Vessels were measured in each eye, and the largest venule and arteriole of each patient were determined and recorded. An intraclass correlation coefficient was used to determine the reliability of this technique²³; the correlation coefficient was 0.90 (95%) confidence interval: 0.75–0.96).

Renal sonography

All renal sonograms were obtained using the Philips IU22 Ultrasound System (Philips Healthcare, Andover, MA, USA) with a 5–8 MHz transducer. The bilateral longitudinal renal length (L), maximal anteroposterior (AP) diameter, and transverse diameter (W) were then measured. To avoid inter-observer errors during scanning and measuring, all scanning was performed by the same sonographer. Kidney volume (KV) was estimated according to the formula $KV = 0.523 \times L \times W \times AP^{24}$. The mean volumes of the right and left kidneys ([Right KV + Left KV] / 2) were then calculated.

Statistical analysis

Statistical analyses were performed using MedCalc Version 11.6 (MedCalc Software, Mariakerke, Belgium). Data are expressed as mean \pm SD or as median [Inter quartile range] where appropriate. Differences were considered to be significant at p < 0.05.

Results

A total of 524 patients were admitted to the department during the study period, of which 227 fulfilled the recruitment criteria. Forty-three infants were recruited, and complete eye and ultrasound scan data were available for 38 infants (12 LBW and 26 NBW; 17 male and 21 female). The mean birth weight for NBW and LBW were 3348 ± 481 g and 2265 ± 183 g respectively. There were no significant differences in the mean gestational age (39.1 ± 1.4 weeks vs. 38.3 ± 1.0 weeks; p = 0.09) and the median post natal age (4.0[3.0-7.0] days vs. 5.0[3.0-7.3] days; p = 0.8) between NBW and LBW infants.

LBW infants had significantly larger retinal arteriole diameter compared with NBW infants (104.2 ± 21.4 vs. 87.0 ± 12.7 μ m; p = 0.004). The retinal venule diameter was also larger in LBW cohort compared to the NBW cohort (146.8 ± 19.5 vs. 128.0 ± 19.5 μ m; p = 0.01). LBW infants also had smaller mean renal volumes (9.3 ± 2.3 vs. 12.2 ± 3.1 ml; p = 0.008). There were negative correlations between retinal arteriolar and venular diameters and renal volumes (r = -0.34, p < 0.05; r = -0.37, p < 0.05, respectively) (Figures 1 and 2). We also compared male and female infants but found no differences in kidney volumes (11.9 ± 3.0 vs. 10.9 ± 3.3 mL; p = 0.34), retinal arteriole diameters (88.5 ± 15.5 vs. 95.0 ± 18.5 μ m; p = 0.27), or retinal venule diameters (129.3 ± 20.4 vs. 137.2 ± 21.0 μ m; p = 0.26).

Discussion

We found that the retinal vessel sizes correlated negatively with renal volume. In addition, retinal vessel diameters (arteriole and venule) were larger in LBW infants. There are many causes of intrauterine growth restriction, but the most common is uteroplacental insufficiency, which results in fetal hypoxia^{25,26}. We postulate that the observed changes in the retinal vessels of our LBW cohort were the result of fetal hypoxia. This finding is most likely to be the result of direct retinal and kidney effects on blood flow, but there may also be secondary changes in the glomeruli of LBW individuals^{7,15}, mediated by vascular endothelial growth factor (VEGF).

Blood flow in the human retina is subject to autoregulation²⁷, a feature of the retina that is similar to that of the kidneys⁹. Retinal circulation is characterized by a low blood flow and a high level of oxygen extraction; the arteriovenous difference in pO_2 is approximately 40%²⁸. Autonomic nerve endings do not extend into the intraocular segments of retinal blood vessels. Therefore, retinal arterial tone is largely regulated by local factors such as local variations in perfusion pressure, pO_2 , pCO_2 , and pH^{28} . Hypoxia plays a major role in the process of angiogenesis in retinal vessels²⁹. The positive regulators of angiogenesis include the members of the VEGF family, angiopoietins, transforming growth factors, epidermal growth factor, vascular endothelial-cadherin, interleukins, and members of the fibroblast growth factor family²⁹.

VEGF is an essential agent of angiogenesis in diseases of the eye²⁹. Of the various types of VEGF present in humans, VEGF-A is the most crucial form for vasculogenesis³⁰. Six different isoforms of VEGF-A have been identified in humans³⁰. Five types of retinal cells have the capacity to produce and secrete VEGF: the retinal pigmented epithelium, astrocytes, Muller cells, endothelial cells, and ganglion cells³⁰. We postulate that placental insufficiency results in fetal hypoxia and a change in the local levels of pO_2 , pCO_2 , and pH. This stimulates the retinal cells to excrete VEGF in order to overcome local hypoxia in the eye. VEGF promotes angiogenesis and vessel dilatation. The dilatation of retinal vessels could also be further enhanced to some extent by an increase in middle cerebral artery flow, which occurs in a hypoxic fetus³¹. Doppler flowmetry data from newborn babies have shown that an increase in blood flow in the central retinal artery³¹.

Extensive changes occur in the circulation of growth-restricted LBW fetuses in response to intrauterine hypoxia. These changes, often referred to as 'redistribution' or the 'brain-sparing effect', reduce vascular resistance in those organs that are essential to fetal survival, such as the brain and coronary arteries^{32,33}. Doppler studies have also shown that fetal hypoxia reduces cardiac output as well as the percentage of cardiac output directed towards the kidneys, thereby resulting in the reduction of renal perfusion, urine production, and amniotic fluid volume in growth-restricted fetuses^{32,33}. Autopsy findings from LBW infants have shown a reduction in glomerular number (and kidney volume) with compensatory glomerulomegaly compared with findings from NBW infants¹⁵. VEGF has also been found to be essential for glomerular and tubular hypertrophy and endothelial cell proliferation in response to nephron reduction³⁴. In the human kidney, the VEGF receptors VEGFR-1 and VEGFR-2 are predominantly expressed on preglomerular, glomerular, and peritubular endothelial cells³⁴. Figure 3 summarizes the relationship between fetal hypoxia, the retinal vasculature, and the glomeruli of the kidney.

The main limitation of this study is the number of subjects recruited. The consent rate was approximately 20%. Participation was voluntary, and parents were not expected to explain why they declined to participate. It is possible that the examination involved in the study might have appeared to be unpleasant for their infants. Another challenge that we faced in the study of newborn term infants was spontaneous retinal hemorrhage, which rendered measurements difficult and inaccurate. Spontaneous retinal hemorrhage is a well-recognized event in healthy term newborns³⁵. The percentage of full-term infants who develop spontaneous retinal hemorrhage at birth ranges from 10% to 30%. The cause of this phenomenon is unknown, but it is not related to the mode of childbirth. Further, it does not require any treatment and resolves naturally.

Conclusion

The larger the kidney (and, by implication, the greater the nephron number), the smaller are the diameters of the retinal arterioles and venules. The increase in retinal vessel diameters is possibly due to compensatory increase in cerebral blood flow in response to fetal hypoxia. Therefore, we suggest that this association shows that chronic hypoxia impairs renal nephrogenesis, as systemic blood flow is restricted in order to maintain cerebral oxygen delivery. Thus, the degree of dilation of the retinal microvasculature could provide an indirect index of renal growth in NBW and LBW infants. We propose that the assessment of the retinal microvasculature is a non-invasive tool to determine at-risk individuals using dilated retinal vessels in LBW infants as a proxy indicator of reduced nephron number.

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Figure 1. The relationship between retinal arteriole diameter and renal volume. Infants that had smaller renal volumes showed larger retinal arteriole diameters. There was also a significant decline in arteriolar diameter as renal volume increased (r = -0.34, p < 0.05). (• for Low birth weight infants)



Figure 2. The relationship between retinal venule diameter and mean renal volume. Infants that had lower birth weights and smaller kidney volumes showed larger retinal venule diameters. There was also a significant decline in venule diameter as renal volume increased (r = -0.37, p < 0.05). (• for Low birth weight infants)



Figure 3. The effect of fetal hypoxia on the renal and retinal microvasculature. Fetal hypoxia causes retinal vessel dilatation and compensatory glomerulomegaly and hyperfiltration. VEGF is believed to be one of the mediators underlying this process. The dilatation of retinal vessels may also be secondary to increased middle cerebral artery flow. ((+) = contributes, \uparrow = increase, \downarrow = decrease)

Relationships between glomerular filtration rate (GFR) and kidney volume in lowbirth-weight neonates

Yogavijayan Kandasamy, Roger Smith, Ian MR Wright, Eugenie R Lumbers Journal of Nephrology, 2012 Oct 3

Abstract

Background: Low birth weight (LBW), defined as birth weight below 2500 g, is an important risk factor for the development of hypertension and renal disease in adult life. LBW is associated with a reduced nephron number, which results in hyperfiltration. The objective of this study was to compare the glomerular filtration rates (GFRs) of LBW and normal-birth-weight (NBW) term infants relative to their kidney volumes.

Methods: Term infants (born after 37 weeks of gestation) who had been admitted to Townsville Hospital's neonatal unit were recruited for this study. Serum Cystatin C was used to calculate GFR. A kidney ultrasound was used to measure renal volume. All assessments were performed during the first week of life.

Results: Data from 39 infants (17 male, 22 female; 13 LBW, 26 NBW) were analyzed. There were no significant differences in the median Cystatin C (1.36 [1.12-1.41] mg/L vs. 1.17 [1.10-1.39] mg/L; p = 0.39) and gestational age. There was no significant difference in the median GFR (53.0[50.8-66.9] mL/min/m² vs. 63.2[51.8-69.5] mL/min/m²; p = 0.39) between LBW and NBW infants, but LBW infants had smaller total renal volume compared to NBW infants (18.0 ± 4.7 mL vs. 24.4 ± 6.2 mL; p = 0.002).

Conclusion: Within 6 days, LBW infants achieved a similar GFR as NBW infants, despite 25% smaller kidney volumes. Thus, the single-nephron glomerular filtration rate must be increased in LBW infants. Prior to this study, it was unclear when hyperfiltration begins, but our results demonstrate that hyperfiltration begins in early life.

Keywords: hyperfiltration, nephron number, glomerulogenesis, Cystatin C, chronic kidney disease

Introduction

Low-birth-weight (LBW) infants (birth weight less than 2500 g), have increased risk of developing chronic kidney disease (CKD) and vascular-related diseases in adulthood (1-5). The association between an adverse fetal or early-postnatal environment and laterlife CKD is compelling and seems to be mediated, at least in part, by impaired nephrogenesis (6, 7). The total number of nephrons is a biological variable that is defined before birth. In humans, approximately 60% of nephrogenesis occurs during the third trimester of pregnancy, continuing up to 34 weeks, and no new nephrons are formed after birth (8-10). The total number of nephrons is thereafter fixed for life.

The best index of renal function is the glomerular filtration rate (GFR). GFR is determined based on the filtration rate of a single nephron multiplied by the total number of nephrons. In 1988, Brenner et al. (11) proposed that a congenital or programmed reduction in nephron number (N_{glom}) could explain why some individuals are susceptible to hypertension and renal injury, whereas others even with excess salt intake or diabetes seem to be relatively resistant to these problems under similar circumstances. When the number of nephrons is diminished, the kidney compensates by increasing the single-nephron GFR (8). According to Brenner's hypothesis, this compensatory mechanism of single-nephron hyperfiltration leads to proteinuria, hypertension, glomerulosclerosis, and ultimately CKD (12). The hyperfiltration hypothesis is supported by animal studies (13) and by autopsy data from hypertensive patients (14). It is possible that the increased risk for CKD is a consequence of a cumulative process resulting from conditions such as diabetes and hypertension, superimposed on a reduced glomerular number and glomerular hypertrophy (8).

On average, humans are believed to have approximately 1 million nephrons per kidney, but nephron number (N_{glom}) in humans can vary from 200,000 to more than 1.8 million (15). Measurement of N_{glom} *in vivo* remains difficult. The most accurate method for determining N_{glom} is through histopathological examination (15). As this is rarely possible in the clinical setting, birth weight and kidney volume are used as a surrogate marker for N_{glom} (9, 16, 17). In a study of 35 neonates (gestational age 36-38 weeks) who died from non-renal causes within 2 weeks of birth, eighteen of whom were LBW, Manalich et al. (9) showed a significant correlation between birth weight and number of glomeruli (r = 0.870, P < 0.0001). Studies conducted in animals (18) and adults (19)

have shown a correlation between renal volume and glomerular number although currently there are none from newborn babies.

Since low-birth-weight infants have fewer nephrons, it is likely that they will be exposed to hyperfiltration (20). Nevertheless, it is still unclear whether hyperfiltration in LBW infants begins in infancy or develops later in life. In this study, we have tested the hypothesis that LBW and NBW infants would, in the first week of life, have similar GFRs despite the smaller kidney volumes of the LWB infants. This would support the theory that nephron hyperfiltration occurs in LBW infants from their first week of life.

Subjects

The study was performed in the Department of Neonatology, The Townsville Hospital, Queensland, Australia. The Department of Neonatology is a tertiary, perinatal center responsible for handling more than 10,000 births each year. Patient recruited into this study was part of a larger study investigating the relationship between birth weight, retinal microvasculature and kidney development. Only data related to renal development is discussed in this paper and other findings (retinal microvasculature) will be discussed in due course. Approximately 25% of the admitted patients are from the Australian Indigenous community. The study commenced in August 2010, and the data presented in this report are based on patients recruited until August 2011. This study was approved by the Townsville Health District Human Research Ethics Committee and was conducted in compliance with good clinical-practice guidelines, institutional review board regulations, and written consent from parents, and it was in accordance with the tenets of the Declaration of Helsinki. Only babies who were born at term (after 37 completed weeks of gestation) were included in the studied sample. Thus, premature babies, as well as infants who needed respiratory support or surgery, infants of diabetic mothers, macrosmic infants (those with birth weight $>90^{\text{th}}$ percentile (21)), and those with syndromes or chromosomal abnormalities were excluded. Babies were classified according to their birth weight (22): those with birth weights less than 2500 g were classified as LBW babies, while those weighing 2500-4499 g were classified as NBW. The mothers were not on any long-term medication and the infants did not receive any nephrotoxic medication.

Methods

All infants recruited into this study underwent ultrasound kidney measurement, venepunture for determining renal function and blood pressure measurements. All assessments were performed in the first week of life.

Kidney measurements

All sonograms were obtained using the Philips IU22 Ultrasound System (Philips Healthcare, Andover, MA, USA) with a 5-8 MHz transducer. Bilateral longitudinal renal length (L), maximal anteroposterior (AP) diameter, and transverse (W) diameter were measured. To avoid inter-observer errors during scanning and measurement, all scans were performed by the same sonographer. Single kidney volume (KV) was estimated according to the formula $KV = 0.523 \times L \times W \times AP$, and total kidney volume (TKV) was calculated as the combined volume of both kidneys (23).

GFR calculation

GFR is the best index of renal function. Serum Cystatin C has been shown in metaanalysis to be superior to serum creatinine as a measure of GFR, since creatinine is influenced by an infant's muscle mass hydration status, and gestational age (24-26). Venous blood was collected through a peripheral venepuncture from each patient on the same day as the renal sonogram was performed. Serum Cystatin C, instead of serum creatinine, was used to calculate and compare GFR. Serum Cystatin C was measured using a commercially available kit (Beckman Coulter, Gentian AS, Moss, Norway) (27). We used a Cystatin C–based prediction equation to calculate the GFR (GFR (mL/min/1.73 m²) = 75.94/[(Cystatin C)^{1.17}] (28).

Birth weight and mean blood pressure were also measured for each baby using a non-invasive blood-pressure recording system (Dash 4000 Monitor, GE HealthCare, Waukesha, WI, USA). The normality of the variables was determined by the D'Agostino-Pearson test (29). Statistical analyses were performed with MedCalc Version 11.6 (MedCalc Software bvba, Mariakerke, Belgium). The data were expressed as mean \pm standard deviation (SD) or as median and interquartile range [IQR]. Student's t-test or Mann-Whitney tests were used where appropriate, with p values < 0.05 considered significant.

Results

A total of 524 patients were admitted to the department during the study period, of which 227 fulfilled the recruitment criteria. Consent was obtained for 43 infants, and complete data were obtained and analyzed from 39 infants (17 male, 22 female; 13 LBW, 26 NBW). There were 13 Australian Indigenous babies in this cohort and 9 of them were LBW. Therefore, the majority of LBW infants in our cohort (9/13) were Australian Indigenous babies. Cystatin C, creatinine, and GFR measurements did not have a normal distribution. Between the LBW and NBW cohorts, infants' mean ages at assessment (5.4 \pm 2.3 days vs. 5.0 \pm 2.3 days; p = 0.63) and blood pressures (59.8 \pm 3.2 mmHg vs. 58.4 ± 6.8 mmHg; p=0.50) were very similar. There were no significant differences in the median Cystatin C (1.36 [1.12-1.41] mg/L vs. 1.17 [1.08-1.39] mg/L; p = 0.39) and gestational age between the two cohorts (38.0±1.0 weeks vs. 39.1±1.4 weeks; p = 0.1). There was also no significant difference in the median GFR calculations (53.0[50.8-66.9] mL/min/m² vs. 63.2[51.8-69.5] mL/min/m²; p = 0.39) between LBW and NBW infants, but LBW infants had smaller total renal volume compared to NBW infants (18.0 \pm 4.7 mL vs. 24.4 \pm 6.2 mL; p = 0.002). The coefficient of variation for the renal volume measurement was 0.289 (28.9%). Figures 1 and 2 show the relationship between birth weight and GFR and the relationship between birth weight and total kidney volume, respectively. There was no correlation between Cystatin C and birth weight.

Discussion

Our study confirmed that LBW infants had significantly smaller kidney volumes and therefore a lower N_{Glom} on average than NBW infants. Despite smaller N_{Glom} , LBW infants had similar GFRs to NBW infants. Since Cystatin C measures the total glomerular filtration, which is the product of the single-nephron GFR x N_{Glom} (8), the single-nephron GFR of LBW infants must be higher than that of NBW infants of the same age. Thus, LBW infants are already hyperfiltering by the first week of life.

Cullen-McEwen et al. studied young mice that had a naturally occurring loss of one allele for glial cell, line–derived neurotrophic factor (GDNF), a loss that results in a reduction in glomerular number of about 30% (13). Histopathological examination of the kidneys from 14-month-old mice showed that while these GDNF heterozygous mice had approximately 30% fewer glomeruli than wild-type mice (9206 \pm 934 vs. 13440 \pm

1275; p < 0.01), GFR and renal blood flow were similar in both groups. Thus, GDNF heterozygous mice apparently maintained normal GFR and renal blood flow despite reduced nephron numbers; in this mouse model, glomeruli were significantly larger and the kidneys engaged in hyperfiltration to maintain GFR in the presence of a reduced nephron endowment.

The incidence of chronic kidney disease (CKD) among indigenous groups in Australia and New Zealand has been shown to exceed that of non-indigenous populations by up to eightfold (30). Hoy et al. demonstrated that up to 35% of the adults in indigenous populations were LBW (31). In another study, Hoy et al. showed that Aboriginal adults had 30% fewer glomeruli than did non-Aboriginal adults (202,000 fewer glomeruli per kidney, or an estimated 404,000 fewer nephrons/individual, p = 0.036) (7, 32). The mean glomerular volume of the Aboriginal group was 27% larger than that of the non-Aboriginal group (p = 0.016). Birth weight, kidney weight, and kidney volume have all been recognized as surrogate measures of N_{Glom}. In Aboriginal populations, glomerular number was significantly correlated with adult height, which suggests a corresponding relationship with birth weight (33), and on average, both birth weight and N_{Glom} are much lower in Aboriginal than in non-Aboriginal people (7). The investigators concluded that the lower nephron number in Aboriginal people increased their susceptibility to CKD.

Should infants who were born with low birth weight be closely followed up and screened regularly for evidence of renal impairment? There no published guidelines on the long-term follow plan for LBW infants. The currently available guidelines in the United Kingdom recommend that the assessment of health status at two years of age be carried out for babies less than 31 weeks gestation or less than a birth weight of 1000 g (34). This guideline focuses on assessment of physical growth, neurodevelopmental outcome, and cognitive function. There are no specific recommendations regarding assessment of renal status (blood pressure, renal function, or urine analysis) when the child during follow-up. Similarly, long-term follow-up plans from other countries focus on developmental outcomes and the incidence of chronic lung disease (35, 36). These follow-ups and assessments (blood pressure, urine for microalbuminuria, renal function) could be incorporated into the existing follow-up guideline. It is unclear if these infants would benefit from specialized nutrition to reduce load on the glomeruli. Perhaps, these

infants might benefit from a nutritional plan similar to that which is prescribed for neonates with chronic renal insufficiency (37).

Prior to this study, it was unclear when hyperfiltration begins, but our results demonstrate that hyperfiltration begins in early life. Long-term follow-up studies are needed to determine the percentage of LBW infants who develop CKD later in life. Hence, we plan to follow our patients and carry out a serial assessment to monitor renal function and renal growth. Though our study is limited by the small number of LBW patients, we plan to continue recruiting such patients in the future and are exploring the possibility of multicenter studies.

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Figure 1 Relationship between birth weight and glomerular filtration (GFR) rate in newborn term infants



Figure 2 Relationship between birth weight and total kidney volume in newborn term infants

Extra-uterine renal growth in preterm infants: Oligonephropathy and prematurity

Yogavijayan Kandasamy, Roger Smith, Ian MR Wright, Eugenie R Lumbers Unpublished manuscript (undergoing peer review)

Abstract

Background and objectives

Nephron number in humans is predetermined during fetal life. The objective of this study was to investigate the effects of preterm birth on postnatal renal growth using renal volume as an estimate of nephron number.

Design, setting, participants, and measurements

This study was conducted over 12 months in a tertiary perinatal center. Preterm babies less than 32 weeks of gestation were recruited and followed up until discharge. Term infants were recruited for comparison. The babies underwent assessments (renal sonography and renal function measurement) at 32 and 38 weeks corrected age (CA). The primary outcome measurement was total kidney volume (TKV) and the secondary outcome was estimated glomerular filtration rate (eGFR).

Results

Forty-four preterm infants and 24 term infants were recruited. At 38 weeks CA, premature infants had lower TKV than term infants $(21.6 \pm 5.7 \text{ vs. } 25.2 \pm 5.7 \text{ ml}; \text{P} = 0.02)$ and a significantly lower eGFR (73.6 [IQR 68.1-77.6] vs. 79.3 [IQR 72.5-86.6] ml·min⁻¹·1.73m⁻²; P = 0.03). There was a significant correlation between TKV and eGFR in premature babies at 32 weeks (Spearman correlation coefficient (rho) = 0.35; P = 0.02), 38 weeks (rho = 0.41; P = 0.01), and in term babies (rho = 0.62; P = 0.002). Multivariate regression model show weight, length, and body surface area are important predictors of TKV at 32 weeks CA.

Conclusions

Premature infants are at increased risk of impaired renal function later in life and could benefit from regular blood pressure and renal function monitoring.

Introduction

The World Health Organization (WHO) estimated that in 2010, 14.9 million, or 11.1% of all births worldwide, were preterm (defined as childbirth occurring at less than 37 completed weeks of gestation) (1, 2). While there has been a slight decline in the preterm birth rate in the US (3), the survival rate of premature babies in emerging economies has improved over time (4, 5), and therefore the number of premature babies surviving to adulthood is anticipated to increase.

Evidence from animal studies (6, 7), human autopsy findings (8, 9), and epidemiology data (10, 11) have indicated that prematurity, independent of birth weight, results in abnormal renal development and predisposes adults to the development of chronic kidney disease (CKD). The nephron number in humans is predetermined during fetal life, as nephrogenesis is completed between 34 and 36 weeks of gestation (7, 12, 13). Preterm birth interrupts normal glomerulogenesis. Poor maternal health (14) coupled with neonatal interventions results in an individual whose nephron endowment is far lower than what nature originally intended (15).

Creatinine clearance and serum Creatinine (SCr) levels are routinely used to monitor renal function in children and adults. The use of serum urea levels (16) and SCr levels to determine renal function in premature babies can be misleading. SCr levels in neonates are: i) subject to negative interference by bilirubin and hemoglobin and positive interference by cephalosporins and ketones (17); ii) SCr levels are not altered until there is a loss of 25–50% of kidney function, and the levels overestimate renal function due to tubular secretion of creatinine at lower glomerular filtration rate (GFR); iii) the SCr level is influenced by an infant's muscle bulk, hydration status, and gestational age (18); iv) at birth, SCr concentrations reflect maternal levels (19); and v) SCr concentration fluctuates in the first weeks of life, where it initially increases during the first 48 hours of extrauterine life, and subsequently reaches a peak and falls thereafter as the GFR rises (19). These limitations together with a delayed increase in SCr after the onset of renal injury make it an unreliable marker in neonates. Therefore, there is currently ongoing research exploring different markers for renal function, and Cystatin C (CysC) is considered to be a very promising candidate (20, 21).

CysC is a cysteine proteinase inhibitor composed of 122 amino acids and has a molecular mass of 13,250 Da (21). It is produced by a housekeeping gene expressed in

all nucleated cells at a constant rate and is freely filtered in the glomerulus with no tubular secretion. Moreover, CysC is completely catabolized by the renal tubules; thus, its plasma level is only dependent on GFR (21). Meta-analyses have shown that CysC is superior to SCr for estimating GFR in children (22, 23). In neonates, there is no correlation between maternal and neonatal serum CysC levels (24). CysC measurements do not differ between male and female infants, and most importantly, there are no gestational age-dependent differences in CysC levels (25-27).

A low nephron number triggers vicious cycle that is associated with glomerular hyperfiltration, glomerular damage, proteinuria, hypertension, and long-term progression to CKD (28, 29). The only accurate method for determining nephron number is to visually count them after autopsy (9, 12). As this is rarely possible in the clinical setting, animal studies (30) and autopsy findings from young infants (31) suggest that kidney volume has a good correlation with total nephron number and could be used as a surrogate measurement for glomeruli number.

Antenatal scans have shown that the normal fetal renal parenchyma grows at a constant and linear rate throughout pregnancy (32). However, to date there are very limited data on extrauterine renal growth in premature babies. Therefore, in this study we postulated that premature infants at term corrected age (CA) would have fewer glomeruli and as a result smaller kidney volumes than term infants. To test this hypothesis, we analysed the effect of prematurity on renal volume in a cohort of premature infants admitted to a tertiary perinatal center for treatment.

Materials and Methods

This study was performed at the Department of Neonatology, Townsville Hospital, Australia, which is a tertiary perinatal center responsible for a region with more than 10,000 births annually. Approximately 2000 births occur at the hospital itself. The neonatal department is divided into an intensive care area (for very ill babies needing respiratory support) and a special care nursery. Recruitment of babies for this study was limited to babies admitted to the department. The study commenced in August 2010, and the recruitment period lasted 12 months. Patients were recruited prospectively and data analysis was carried out retrospectively upon completion of the study period. The Townsville Health District Human Research Ethics Committee approved this study, which was conducted in accordance with the tenets of the Declaration of Helsinki.
Written parental consent was obtained from the parents of all infants who participated in this study.

Patients

Preterm babies at less than 32 weeks of gestation admitted to the neonatal intensive care unit during the study period were eligible to participate in this study. Patients with congenital abnormalities or syndromes were excluded. Preterm babies were recruited and followed up until discharge. Once recruited, the patients underwent a first assessment (renal sonography and renal function measurement) at 32 weeks CA and a second assessment at 38 weeks CA (renal sonography, renal function, and blood pressure measurements). CA for a premature baby is defined as: [CA = age at birth (gestational age in weeks) + postnatal age in weeks] (33). For example, a premature baby born at 28 weeks of gestation will be 32 weeks CA after 4 weeks of postnatal life and 38 weeks CA after 10 weeks of postnatal life.

Term babies (37 completed weeks of gestation) with a birth weight that was appropriate for gestational age (AGA; weight between 10^{th} -90th percentile) (34) admitted to the special care nursery during the same study period were also recruited for this study. These infants were admitted for non-life threatening neonatal problems, such as neonatal jaundice, risk of sepsis, and transient tachypnea of the newborn (TTN). Term babies with congenital abnormalities or syndromes, infants requiring respiratory care, infants of diabetic mothers, small for gestation age (SGA; weight < 10^{th} percentile) (34), and infants whose parents did not provide written consent were excluded from the study. All term infants underwent renal sonography, renal function assessment, and blood pressure measurements within the first week of life. All three measurements were obtained on the same day.

Anthropometric and blood pressure measurements

All anthropometric and blood pressure measurements were carried out by neonatal nurses. For all newborn premature infants, birth weight was measured using an electronic weighing scale built into the incubator (Giraffe Incubator, General Electrics Healthcare, Laurel, MD, USA). All other body weight measurements were performed with an electronic infant weighing scale (Seca 727 Electronic baby scale, Seca Deutschland, Hamburg, Germany). For measurements, each infant was placed at the center of the scale tray without any clothes/diapers. One nurse ensured that the infant

was safely secure on the scale, while a second nurse recorded the weight. The weight was recorded to the nearest 0.01 kg. The infants were then repositioned for a second time on the scale and a second measurement was recorded. If the first two readings exceeded a 0.1 kg difference, then a third measurement was conducted. The closest two weights were averaged.

The length measurement (head to toe) was carried out with the infant lying supine and with infant's body, hips, and knees straightened. Measurements were taken twice and then averaged. Mean blood pressures were measured for each infant at 32 and 38 weeks for premature babies and once for term infants. The blood pressure was obtained using a non-invasive blood pressure recording system (Dash 4000 Monitor; GE HealthCare, Waukesha, WI, USA). An infant cuff was applied to the right upper arm with the infant calm and lying supine. Three successive BP recordings were taken at 2 min intervals and the mean was calculated (35).

Kidney ultrasonography

All renal ultrasounds were obtained using the Philips IU22 Ultrasound System (Philips Healthcare, Andover, MA, USA) with a compact (small footprint) curved linear 5-8 MHz frequency transducer. To avoid inter-observer bias during scanning and measuring, the same neonatal sonographer performed all examinations. The sonographer was also blinded to the clinical information of the patients. Renal length (L), anteroposterior diameter (AP), and transverse diameter (W) were measured for both kidneys. Kidney volume (KV; ml) was calculated according to the following formula: $KV = (\pi/6 \times L \times W \times AP)$ (36). The mean volume of the right and left kidneys [(Right KV + Left KV)/2] and total KV (Right KV + Left KV) were also calculated.

Renal function and GFR calculations

Venous blood was collected through a peripheral venipuncture for serum SCr and Cys C measurements from each patient on the same day as the renal sonogram was obtained. Serum Cys C was measured using a commercially available kit according to the manufacturer's instructions (Beckman Coulter, Gentian AS, Moss, Norway). A Creatinine/Cys C-based prediction equation was then used to calculate the estimated glomerular filtration rate (eGFR) as follows: eGFR (ml·min⁻¹·1.73 m⁻²) = [507.76 x $e^{0.003 \text{ x height}}$]/[CysC^{0.635} x SCr^{0.547}](**37**).

Outcome measures

The primary outcome measured in this study was total kidney volume (TKV). The TKV was assessed at 32 weeks CA and 38 weeks CA for premature babies and within the first week of life for term infants. The secondary outcome was a comparison of eGFR.

Statistical methods

Data from a previously published study show that the TKV in term infants is 9.6 ± 2.6 ml (38). Studies in adults who were born prematurely show that their kidney volume is 15% lower than that of adults who were born at term (39); thus, for a study with $\alpha = 0.05$ (2-sided) and $\beta = 80\%$, 26 patients were needed for each cohort.

The normality of the variables was determined by the D'Agostino-Pearson test (40). The results were expressed as the means \pm standard deviation (SD) for continuous, normally distributed data and as median (interquartile range [IQR]) for continuous, non-normally distributed data. Comparisons of means of normally distributed data were made using paired/unpaired *t*-tests, and Mann-Whitney or Kruskall-Wallis tests were used for non-normally distributed data. The degree of association between TKV or eGFR and the variables of interest were assessed using Pearson's correlation for continuous, normally distributed data. One-way ANOVA and linear regression analysis was performed as appropriate. Multivariate models were constructed using stepwise backward regression. Regression coefficient values with 95% confidence intervals (CI) were also calculated. A *P* value of < 0.05 was considered statistically significant. Statistical analyses were performed using Stata Version 11.0 statistical software (StataCorp, College Station, TX, USA).

Results

Figure 1 summarizes the number of patients available for recruitment and the actual number recruited. Forty-nine preterm babies were recruited; two babies died and 3 were transferred back to regional hospitals. The deceased preterm infants were also small for their gestational age (SGA, birth weight < 10^{th} percentile). Data from 44 preterm babies were used for analysis (18 females, 26 males). Twenty-four term babies (14 males, 10 females; AGA) were also recruited. The mean gestational age for premature infants was 28.0 ± 2.4 weeks, with a mean birth weight of 1133 ± 339 g. The median gestational age

for the term babies was 38.7 weeks [IQR 37.1–39.4 weeks], and the mean age was 5.0 ± 2.3 days.

Table 1 summarizes the clinical data for preterm and term infants. At 38 weeks CA, premature infants had smaller TKVs than term babies $(21.6 \pm 5.7 \text{ vs. } 25.2 \pm 5.7 \text{ ml}; \text{P} = 0.02)$ (Figure 2). They also had smaller body weights compared to term infants $(3416 \pm 433 \text{ vs. } 2566 \pm 406 \text{ g}; \text{P} < 0.001)$. Preterm infants at 38 weeks CA had significantly higher levels of Cys C (1.41 [IQR 1.28–1.58] vs. 1.18 [IQR 1.1–1.40] mg/L; P = 0.03) and a lower eGFR (73.6 [IQR 68.1-77.6] vs. 79.3 [IQR 72.5-86.6] ml·min⁻¹·1.73 m⁻²; P = 0.03) compared to term babies. There was no difference in mean blood pressure measurements between the preterm infants at 38 weeks CA and term babies. There were also no significant differences in the mean TKV of male and female preterm infants at 32 weeks CA ($15.2 \pm 4.6 \text{ vs. } 13.9 \pm 5.2 \text{ ml}$; P = 0.39) and at 38 weeks CA ($22.1 \pm 5.1 \text{ vs. } 20.9 \pm 6.7 \text{ ml}$; P = 0.48). There were also no significant differences at 32 weeks CA ($7.6 \pm 2.5 \text{ vs. } 7.2 \pm 2.4 \text{ ml}$; P = 0.39) and 38 weeks CA ($11.1 \pm 3.1 \text{ vs. } 10.7 \pm 2.9 \text{ ml}$; P = 0.50).

We carried out a correlation analysis for TKV and eGFR using Spearman's rank correlation coefficient (rho) and found a significant correlation in premature babies at 32 weeks CA [rho = 0.35 (95% CI 0.07-0.58); P = 0.02], 38 weeks CA [rho = 0.41 (95% CI 0.12-0.64); P = 0.01] and in term babies [rho = 0.62 (95% CI 0.27-0.82); P = 0.002]. Using simple bivariate regression analysis, we found that TKV in preterm babies increased with heavier body weight, but there was no significant association for term infants (Figure 3). A multivariate regression model showed that weight, length, and body surface area were significant predictors of kidney volume at 32 weeks (adjusted R² 0.33, P < 0.001), but not at 38 weeks (Tables 2 and 3). There were no significant correlations noted between blood pressure and kidney volume in both preterm and term babies.

The majority of the preterm infants (40/44) and term infants (21/24) in our study were fed with breast milk. None of the preterm babies developed kidney failure.

Discussion

In our cohort, kidney volume was lower in premature infants than in term infants. Premature babies also had a lower eGFR compared to term infants. We found that total kidney volume had a positive correlation with eGFR in both premature and term babies, which supports the hypothesis that kidney volume in young infants can be used as a surrogate marker of nephron number. Weight, length, and body surface area were significant predictors of kidney volume at 32 weeks, but not at 38 weeks. The reason for this is unclear, and it is likely that extrauterine renal growth depends on other factors, such as the administration of antenatal steroids (41) to pregnant women with preterm contractions, placental insufficiency (42), congenital renal abnormalities, administration of aminoglycosides (43) in the neonatal unit, treatment of Patent Ductus Arteriosus (PDA) with nonsteroidal anti-inflammatory drugs (NSAIDS) (44), and infant nutrition (16). In our cohort, the mothers of all premature babies received at least one dose of antenatal steroids (betamethasone) before they went into labor. There was no maternal history of renal disease, and none of the preterm babies had renal abnormalities or were treated for renal failure. We do not routinely administer NSAIDS for the treatment of PDA.

The American Academy of Pediatrics' current recommendations on follow-up of high-risk premature infants, while very comprehensive (45), is primary focused on issues such as neurodevelopment outcomes and respiratory complications. Current guidelines and follow-up data from other countries (46, 47) have a similar focus. However, there are no recommendations regarding the need for routine blood pressure, renal function, and urine analysis monitoring in these patients once discharged from the neonatal intensive care units.

There were several limitations to our study. First, only a small proportion of eligible patients were recruited for this study (term: 20%; preterm: 44%). Participation in this study was voluntary and parents were not required to give an explanation for declining. However, the few that did voluntarily provide an explanation indicated that they were not keen on their baby being subjected to venepuncture and other investigations that were not part of routine care. Some of the parents of term infants were also not keen or were unable to return to the hospital for the tests and scan.

We were unable to compare kidney growth in SGA and AGA premature babies. There were two deaths in our cohort, and both were SGA premature babies. The mortality rate among SGA premature babies is approximately 2.5-3 times greater than that of AGA premature babies (48). The study was also limited by the fact that we did not carry out weight measurements for premature babies and term babies using the same measuring scale. We used a weighing scale built into the neonatal incubator for

measuring birth weight and then used an electronic weighing scale for subsequent measurements. We did not want to risk the development of hypothermia in the newborn premature babies, which is associated with increased mortality and morbidity (49).

All anthropometric and blood pressure recordings were carried out by neonatal nurses. It was not possible to assign the same person to carry out the measurements at the time of admission to the neonatal units, which can be unpredictable. In addition, we were also unable to carry out analysis to determine the intra-and inter-measurement estimated errors.

Conclusion

This study showed that premature babies have a smaller kidney volume and lower eGFR compared to term infants. We also demonstrated that eGFR has a good correlation with kidney volume, which supports the concept that kidney volume is a surrogate marker for nephron number in neonates. We propose that ex-premature infants would benefit from regular blood pressure and renal function monitoring.

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	Preterm infants			
	1 st assessment	2 nd assessment	I erm infants	
Number of infants	44	44	24	
Gestation (weeks)	32.2 [31.8-32.2]	38.0 [37.7-38.1]	38.7 [37.1-39.4]	
Mean Weight (g)	1447 ± 250	2566 ± 406	3416 ± 433	
Mean length (cm)	38.9 ± 4.0	45.0 ± 3.2	50.1 ± 2.1	
Size for gestational age	10 th -50 th percentile	10 th percentile	50 th percentile	
(34)				
$BSA(m^2)$	0.13 ± 0.02	0.18 ± 0.02	0.22 ± 0.02	
Mean Blood Pressure	41.3 ± 2.1	59.8 ± 10.0	57.9 ± 6.8	
(mmHg)				
eGFR (ml·min ⁻¹ ·1.73 m ⁻²)	67.2 [IQR 55.3-79.2]	73.6 [IQR 68.1-77.6]	79.3 [72.5-86.6]	
Mean Renal length (cm)	3.6 ± 0.3	4.2 ± 0.3	4.5 ± 0.4	
Mean Renal width (cm)	2.0 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	
Total Renal Volume (ml)	14.5 ± 4.7	21.6 ± 5.7	25.2 ± 5.7	
Serum creatinine (µmol/L)	37.8 ± 9.8	30.6 ± 2.3	37.8 ± 16.5	
Cystatin C (mg/L)	1.40 [IQR1.2–1.6]	1.41 [IQR 1.28–1.58]	1.18 [IQR 1.1–1.40]	

Table 1. Comparison between term and premature infants

BSA = Surface Area = $W^{0.5378} \times L^{0.3964} \times 0.024265$ (50)

Table 2. Multivariate association between kidney volume at 32 weeks with other variables (adjusted $R^2 = 0.33$, P < 0.001)

Variable	Coefficient	Standard Error	95% Confidence Interval	Р
Weight at 32 weeks (g)	0.2	0.1	0.03-0.36	0.02
Length at 32 weeks (cm)	5.3	2.3	0.64-9.97	0.03
BSA at 32 weeks (m ²)	-4035.2	1733.6	-7529.1541.2	0.02

BSA = Body surface area

Table 3. Multivariate association between kidney volume at 38 weeks with other variables (adjusted $R^2 = 0.14$, P < 0.03)

Variable	Coefficient	Standard Error	95% Confidence Interval	Р
Weight at 38 weeks (g)	0.0	0.1	-0.09-0.19	0.5
Length at 38 weeks (cm)	1.9	3.0	-4.22-8.12	0.5
BSA at 38 weeks (m ²)	-1102.0	1834.5	-4812.672608.58	0.6

BSA = Body surface area



Figure 1. Selection of preterm and term babies for the study



Figure 2. Box plot showing a significant difference in total kidney volume between preterm (32 weeks and 38 weeks) and term babies (one-way ANOVA; p<0.001, degree of freedom = 2).



Figure 3. Linear regression graphs showing the relationship between total kidney volume and body weight in premature and term infants.

Chapter Four – Conclusions and Future Direction

In utero insults that result in LBW infants are now well recognised as risk factors contributing to the development of vascular-related diseases in adulthood (30). There is increasing evidence to suggest that microcirculatory pathology forms the mechanistic link between fetal insult and the adult manifestation of illness. The retina provides an opportunity for *in vivo* investigation of human microcirculation, and changes in the retinal vessels have been identified in some individuals who had LBW as infants and later developed hypertension, ischemic heart disease, stroke, and renal disease (27-30).

From this study, we were able to determine for the first time the normal value for retinal vessel diameter in newborn term babies, a clinical information previously unknown (41). This study also demonstrated that retinal venule diameter is larger when compared to retinal arteriole diameter (41). The arteriovenous ratio, an important risk factor measurement, is smaller in neonates compared to children and adults indicating the retinal arteriole diameter increase at a different pace compared to retinal venule. Sex did not influence the retinal microvasculature size in infants(41). On the other hand, retinal venules and arterioles in LBW babies are larger than those of normal-birthweight babies (42). We postulate that the difference observed in our study was due to *in utero* pathophysiological changes that occurred in the cerebral circulation of growth-restricted fetuses.

Our study also demonstrated that LBW term babies have smaller kidney volume (and, by implication, a smaller nephron number) when compared to term normal weight infants (43, 44). This study demonstrated that the larger the kidney, the smaller the diameters of the retinal arterioles and venules (43). Thus, the degree of dilation of the retinal microvasculature could provide an indirect index of renal growth. The assessment of the retinal microvasculature could potentially be used as a non-invasive and a proxy indicator of reduced nephron number. This study also demonstrated that within 6 days, LBW infants achieved a similar eGFR as normal-birth-weight infants, despite their 25% smaller kidney volumes (44). We postulate that the single-nephron glomerular filtration rate must be increased and kidneys of these infants are hyperfiltrating. Prior to this study, it was unclear when hyperfiltration begins, but these results demonstrate that hyperfiltration begins in early life.

When we investigated premature infants, we found that premature babies have smaller kidney volume and body weight at term-corrected age than full-term babies. This study also demonstrated that premature babies have a lower eGFR. We found that total kidney volume had a positive correlation with eGFR in both premature and term babies.

A recently published study showed that preterm very-low-birth-weight infants develop elevated systolic blood pressure, dysfunctional renal auto regulation and hyperfiltration by the age of one year (45). Future research would involve following up the babies into adulthood with regular monitoring of blood pressure, renal volume, renal function and serial retinal microvasculature measurements. Future studies would also need to investigate the effect of hyperfiltration and the possible need of intervention to delay or to prevent chronic kidney diseases.

Appendices

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September 13, 2012

VIA EMAIL & REGULAR MAIL

Dr. Yogavijayan Kandasamy MD 11 Beylando Bend, Douglas Queensland 4814, Australia

Re: Use of *Star Wars* quote in thesis entitled, "The Relationship Between Birth Weight, Prematurity, Renal Volume and Retinal Micro Vasculature in Premature and Low Birth Weight Babies"

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