Indices of renal tubular function in perinatal asphyxia

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Abstract

***Aims*—To determine and compare two urinary indices of renal tubular function, *N*-acetyl-glucosaminidase (NAG) and β_2-microglobulin (β_2M), in healthy term neonates and babies with perinatal asphyxia.**

***Methods*—In a prospective case–control study using asphyxiated (n=35) and normal control (n=55) infants, urinary NAG and β_2M were assayed at 24–48 hours of life, 4–6 days, and 4–6 weeks.**

***Results*—NAG and β_2M were significantly increased at 24–48 hours and 4–6 days in the asphyxiated infants compared with the controls. Increased NAG values reflect the degree of perinatal asphyxia more than do β_2M. Gentamicin also increased NAG excretion, but to a lesser extent than did perinatal asphyxia.**

***Conclusions*—NAG (+/- β_2M) may be a useful marker of perinatal asphyxia. Urinary NAG concentrations correlate with the severity of perinatal asphyxia.**

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Keywords: perinatal asphyxia; renal tubular function; *N*-acetyl-glucosaminidase

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Perinatal asphyxia causing organ damage is an important neonatal problem. Its study is challenging because it is difficult to measure. The kidney is very sensitive to ischaemic damage.[1] Fetal and neonatal "asphyxia" are the main causes of transient renal impairment or acute renal failure in neonates.[2 3]

Urinary concentrations of *N*-acetyl-glucosaminidase (NAG) and β_2-microglobulin (β_2M) are sensitive indices of renal tubular function. NAG is a lysosomal enzyme present in proximal tubular cells.[4] Its molecular weight precludes filtration at the glomerulus but it is rapidly cleared from the circulation by the liver. Increased urinary excretion is a consequence of renal tubular cell breakdown.[5] β_2M is a low molecular weight protein, freely filtered through the glomerulus, but almost totally reabsorbed in the proximal tubules.[6] Urinary β_2M concentration increases with degree of tubular dysfunction.

The objective of this study was to compare concentrations of urinary NAG and β_2M in a population of asphyxiated and normal term neonates over the first 4 to 6 weeks of life.

Methods

This prospective case–control study compared 35 term babies with perinatal asphyxia (group 1) with a group of 55 normal term infants (group 2). Informed consent was obtained from the parents of all participants. Single "spot" urine samples were collected using a standard paediatric urine bag at 24 to 48 hours, 4 to 6 days, and 4 to 6 weeks of life. Group 1 subjects had urine samples tested daily during the period of hospital admission for the presence of haematuria and routine serum biochemistry (including creatinine concentrations) performed at least daily for the first week.

Group 1 comprised 35 consecutive term babies with perinatal asphyxia transferred from maternity hospitals to a level 3 neonatal intensive care unit at a paediatric hospital (Princess Margaret Hospital for Children, Perth, Western Australia). Perinatal asphyxia was defined as a 5 minute Apgar score of 5 or less plus fulfilment of the diagnostic criteria of post-hypoxic/ischaemic encephalopathy according to the clinical staging system of Sarnat[7] (table 1). Each case was assigned a Sarnat score, reflecting the degree of severity. Prenatal signs of fetal distress, including meconium stained liquor and fetal heart rate abnormalities, were sought to confirm the clinical impression, as were umbilical cord blood gas values if available. Cases were retrospectively reassessed after discharge or post mortem examination and excluded if pathology other than perinatal asphyxia was suggested.

Group 2 comprised normal term infants (n=55) recruited from a suburban maternity hospital (Woodside Maternity Hospital, East Fremantle, Western Australia). Subjects in this group had 5 minute Apgar scores of more than 6, normal behaviour over the first week of life, and were classified as normal on routine clinical examination.

To identify the possible influence of amino glycoside antibiotics on results for group 1, urine samples at 24 to 48 hours of life were obtained and analysed for NAG from 13 babies consecutively admitted with transient tachypnoea of the newborn (TTN), but no evidence of perinatal asphyxia, and 12 further infants with perinatal asphyxia (as defined before). These subjects form groups 3a and 3b, respectively. Infants in both groups were routinely treated with antibiotics (gentamicin and ampicillin) for at least the first 24 hours of life, at which time urine samples were collected. Gentamicin concentrations were measured and those with raised values excluded from analysis. Further data on the subjects are given in table 2.

Samples were frozen at −20 to −70°C, until assay, when the pH was adjusted to 6–8.

Table 1 Sarnat score: clinical staging of posthypoxic encephalopathy

Factor	*Stage 1*	*Stage 2*	*Stage 3*
Consciousness	Alert	Lethargic	Coma
Tone	Normal	Hypotonic	Flaccid
Tendon reflexes	Increased	Increased	Depressed
Myoclonus	Yes	Yes	No
Suck	Active	Weak	Absent
Moro response	Increased	Incomplete	Absent
Grasp reflex	Normal	Increased	Absent
Oculocephalic	Normal	Increased	Depressed
Pupils	Dilated	Constricted	Variable/fixed
Respiration	Regular	Periodic/variable	Apnoea
Heart rate	Increased/normal	Bradycardia	Bradycardia
Seizures	No	Common	Uncommon
EEG	Normal	Periodic or paroxysmal	Periodic or isoelectric

Table 2 Clinical data on neonates studied

Group	*Gestational age (weeks)*	*Birthweight (g)*	*5 minute Apgar score*
1 (n=35) Perinatal asphyxia	36-42 (39)	*1720-4970 (3307)	1-4 (3)
2 (n=55) Healthy full term	37-42 (40)	2480-4990 (3584)	9-10 (9)
3a (n=13) Transient tachypnoea	36-40 (38)	2200-3560 (3110)	7-9 (9)
3b (n=12) PA paired with TTN	36-42 (39)	2575-4045 (3440)	1-4 (3)

All data are presented as mean and range; * Two infants were small for gestational age, one was a twin.

Urinary β_2M was measured using a competitive enzyme immunoassay (T-Cell Diagnostics Inc., Cambridge, MA, USA). Specimens containing β_2M were incubated with rabbit anti-human β_2M antibody and with horseradish peroxidase labelled human β_2M to compete for a limited number of binding sites. During incubation, the antibody simultaneously bound to the microtitre plate, which had been coated with an antigen to anti-β_2M. Unbound conjugate was removed by washing, and the remaining enzyme-labelled antigen incubated with 3,3'-5,5'-tetramethylbenzidine substrate. The reaction was terminated by addition of 2 molar H_2SO_4, and the absorbency (inversely proportional to the concentration of β_2M in the sample) was measured at 450 nm. Results were expressed as milligrams per litre.

NAG was determined colorimetrically by measuring the release of 3-cresol purple from the substrate 3-cresolsulphonphthaleinyl-*N*-acetyl-B-D-glucosaminide (Boehringer Mannheim, Germany). Results were expressed as International Units per millimole of creatinine. Serum and urine creatinine concentrations were measured using a dry chemistry analyser, Kodak Ektachem 500 (Eastman Kodak, Rochester, New York). Haematuria was assessed using standard dipstick reagent strips (Multistix, Bayer Diagnostics, Victoria).

Statistical analysis was performed using INSTAT (Instant Biostatics, version 2). Mean, median, standard deviations, and 95% confidence limits are reported and were tested using the Kruskal-Wallis non-parametric ANOVA test and the Mann-Whitney U test, giving a two tailed P value. Non-parametric statistics were applied as logarithmic transformation failed satisfactorily to normalise the data.

The study was approved by the Research and Ethics Committee of Princess Margaret Hospital for Children.

Results

The postnatal progression in urinary NAG and β_2M concentrations are shown in table 3. Initially raised concentrations of both NAG and β_2M in the perinatal asphyxia group returned to control group values by 4 to 6 weeks of life.

Table 4 shows the effect of gentamicin on urinary NAG at 24 to 48 hours of life in groups 2, 3a, and 3b. Median NAG concentrations for TTN (group 3a) and perinatal asphyxia (groups 1 and 3b) subjects differed significantly from those of normal controls (group 2) ($P<0.0001$). The perinatal asphyxia group had significantly higher values than did the TTN group ($P = 0.02$).

The correlation between median urinary NAG concentrations at 24 to 48 hours of life and the degree of perinatal asphyxia, as assessed by the Sarnat score, is shown in table 5. There was an increase in mean urinary NAG excretion with increasing severity of perinatal asphyxia. Median values for the control group differed significantly from those of Sarnat 1 ($P = 0.0004$), Sarnat 2 ($P<0.0001$), and Sarnat 3 ($P = 0.0026$) groups. There was also a positive correlation which was not significant for NAG at 4 to 6 days; this was not seen with β_2M at 24 to 48 hours or 4 to 6 days (data not shown).

Dipstick testing for haematuria in the perinatal asphyxia group over the first 4 days of life revealed "large" blood on at least one occasion in 14/32, "trace" or "small" in 5/32 and no blood in 13/32 infants. No information was recorded for three infants.

The mean serum creatinine concentration for the perinatal asphyxia group was 101.1

Table 3 β_2M and NAG values over time

	β_2M			*NAG*		
	24-48 hours	*4-6 days*	*4-6 weeks*	*24-48 hours*	*4-6 days*	*4-6 weeks*
Control group						
Number	35	34	25	55	52	41
Mean	1.17	1.55	0.34	1.89	3.50	2.57
SD	1.33	1.26	0.47	1.21	3.36	1.86
Median	0.66	1.60	0.13	1.60	2.60	2.10
95% CI	0.71-1.62	1.12-1.99	0.15-0.53	1.56-2.22	2.56-4.43	1.89-3.16
PA group						
Number	34	24	10	35	23	10
Mean	7.85	10.60	0.34	28.84	29.31	2.20
SD	11.57	14.33	0.51	65.56	85.57	1.27
Median	3.88	3.69	0.13	9.60	8.30	1.85
95% CI	3.81-11.90	4.59-16.61	−0.02-0.70	3.30-48.38	−7.70-66.31	1.29-3.11
P value	< 0.0001	< 0.0005	0.985	< 0.0001	< 0.0001	0.469

Table 4 Gentamicin effects on 24-48 hour NAG concentrations

	Control (group 2)	*TTN (gentamicin) (group 3a)*	*PA (gentamicin) (groups 1 and 3b)*
Number	55	13	47*
Mean	1.89	5.03	22.40
SD	1.21	2.78	56.93
Median	1.60	4.00	9.60
95% CI	1.56-2.22	3.35-6.71	5.68-39.14

* Includes 12 samples collected with TTN group.

Table 5 Sarnat score and 24-48 hour NAG concentrations

	Control	*Sarnat 1*	*Sarnat 2*	*Sarnat 3*
Number	55	12	15	7
Mean	1.89	9.28	20.71	68.54
SD	1.21	9.08	14.30	144.56
Median	1.60	4.50	20.40	4.50
95% CI	1.56-2.22	3.52-15.05	12.79-28.63	−65.16-202.24

μmol/l at 24 to 48 hours (SD 50.7, n=33, range 41–252) and 63 μmol/litre (SD 22.3, n=21, range 45–121) at 4–6 days of life.

Discussion

Traditionally, assessment of perinatal asphyxia has relied on a combination of clinical observations, such as Apgar score, and measurement of systemic indices of tissue ischaemia, such as serum creatinine. There are weaknesses in such methods.

Clinical scoring systems generally reflect neurological state (encephalopathy) which may be influenced by factors other than perinatal asphyxia, such as metabolic and chromosomal disorders. Objective measurements, such as serum creatinine, are insufficiently sensitive to be generally useful. The first form of assessment lacks specificity in the context of perinatal asphyxia; the latter lacks sensitivity.

Perlman[8] compared several systemic manifestations of asphyxia, including oliguria, serum creatinine, seizures, and cranial ultrasound abnormalities and found that urinary β_2M was the most sensitive index of systemic organ injury caused by perinatal asphyxia. Fernandez *et al*[9] found that urinary β_2M was more sensitive and specific than "usual tests of renal function" after perinatal asphyxia. Kojima[2] showed raised NAG in a group of asphyxiated infants over the first week of life, a finding which agrees with our results.[10]

Our data suggest that both urinary NAG and β_2M are significantly increased in asphyxiated compared with normal term neonates over the first 24 to 48 hours of life. At 4 to 6 weeks there is no statistical difference between the groups, suggesting resolution of hypoxic injury to the renal tubules over this time.

Serum creatinine concentrations have been considered to be indices of perinatal asphyxia. But interpretation is complicated by the fact that for at least the first 48 hours of life, serum creatinine, to a significant extent, reflects maternal concentrations.[11] Raised values were found in infants with perinatal asphyxia in our study at 24 to 48 hours of life, although the range was wide. The mean value at 4 to 6 days falls within the newborn reference interval. Renal tubular dysfunction is known to occur more commonly than glomerular dysfunction in perinatal asphyxia,[5] further suggesting a relative insensitivity of serum creatinine compared with indices of tubular function such as urinary β_2M and NAG.

Haematuria has been associated with perinatal asphyxia. Tack[12] found chemical haematuria in 71% of 140 sick term and premature infants, most of whom were asphyxiated. However, a significant number of non-asphyxiated babies also had haematuria. In our group of term neonates with perinatal asphyxia, 13/32 had no haematuria, including one severely asphyxiated infant who died on the fourth day of life, and 18/32 who had no more than "small" blood on any occasion. It should also be noted that chemical methods for detecting haematuria are non-specific, picking up myoglobin and haemoglobin. In our babies the presence of haematuria on dipstick testing is relatively insensitive for identifying perinatal asphyxia.

None of the infants studied received tolazoline or indomethacin, both of which may injure the newborn kidney. However, use of gentamicin which may be nephrotoxic introduces a possible confounder,[13] although blood concentrations were within the therapeutic range in all subjects. Our data show an increase in urinary NAG at 24 to 48 hours in both groups treated with gentamicin (perinatal asphyxia and TTN groups) but a greater increase in the former. The results show a positive correlation between NAG and Sarnat score at both 1 to 2 and at 4 to 6 days of life. All babies received similar treatment with gentamicin but NAG rose in proportion to the severity of neonatal asphyxia, suggesting that the antibiotic effect is not the predominant contributory factor.

An objective and semiquantitative index of perinatal asphyxia would be a helpful addition to current techniques for assessing asphyxiated or encephalopathic neonates. In certain circumstances clinical scoring systems are influenced by factors other than perinatal asphyxia without an ability within the scoring system to identify possible aetiologies. With the development of "rescue therapies" for use in perinatal asphyxia, it may be useful to be able to predict at a relatively early stage which infants are likely to benefit most from these treatments that may carry significant risks.

Both β_2M and NAG are sensitive indices of renal tubular dysfunction in asphyxiated term neonates. Their values probably reflect systemic organ damage produced by an asphyxial insult. Urinary NAG and β_2M may be useful markers of asphyxia in addition to clinical indicators such as Apgar and Sarnat scores.

Our data suggest that unlike β_2M, urinary NAG increases proportionate to the degree of encephalopathy/asphyxia, as defined by Sarnat score, an association present at both 24 to 48 hours and 4 to 6 days of life. NAG is also more attractive than β_2M due to its lower cost.

Measurement of urinary NAG in the first days of life may assist in the diagnosis and assignment of prognosis to an asphyxiated infant, although further studies are required to test these findings.

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1 Talosi G, Streitman K, Suranyi A, Horvath I, Pinter S. Renal injury in perinatal hypoxia: changes of renal function and ultrasonography. *Pediatric Nephrol* 1996;**10**:C156.
2 Kojima T, Kobayashi T, Matsuzaki S, Iwase S, Kobayashi Y. Effects of perinatal asphyxia & myoglobinuria on development of acute neonatal renal failure. *Arch Dis Child* 1985;**60**:908-12.
3 Karlowicz MG, Adelman RD. Nonoliguric and oliguric acute renal failure in asphyxiated term neonates. *Pediatric Nephrol* 1995;**9**:718-22.
4 Watanabe K, Kojima T, Fukuda Y, Ohbayashi K, Kobayashi T, Iwase S, Kobayashi Y. Reliability of urinary N-acetyl-B-D-glucosaminidase as an indicator of renal tubular damage in neonates. *Biol Neonate* 1987;**52**:16-21.
5 Roberts DS, Haycock DB, Dalton RN, Turner C, Tomlinson P, Timmler L, Scopes JW. Prediction of acute renal failure after birth asphyxia. *Arch Dis Child* 1990;**65**:1021-8.
6 Tsukahara H, Yoshimoto M, Saito M, Sakaguchi T, Mitsuyoshi I, Hayashi S, *et al*. Assessment of tubular function in neonates using urinary Beta-2-microglobulin. *Pediatric Nephrol* 1990;**4**:512-14.
7 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. *Arch Neurol* 1975;**33**:669-705.
8 Perlman JM, Tack ED, Martin T, Shackelford G, Amon E. Acute systemic organ injury in term infants after asphyxia. *Am J Dis Child* 1989;**143**:617-20.
9 Fernandez F, Barrio V, Guzman J, Huertas MD, Zapatero M, de Miguel MD, Mallol J. Beta-2-microglobulin in the assessment of renal function in full term neonates following perinatal asphyxia. *J Perinat Med* 1989;**17**:453-9.
10 Willis FR, Summers J, Minutillo C, Hewitt I. Urinary indices of renal dysfunction in neonatal asphyxia. European Society for Paediatric Research Annual Meeting, Edinburgh, 1993. *Pediatr Res* 1994;**35**:281.
11 Mehta KP. Neonatal renal failure. *Indian Pediatrics* 1991;**28**:7-9.
12 Tack ED, Perlman JM, Robson AM. Renal injury in sick newborn infants: a prospective evaluation using B2-microglobulin concentrations. *Pediatrics* 1988;**81**:432-9.
13 Mathew TM. Drug induced renal disease. *Med J Aust* 1992;**156**:724-8.