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Neonatal renal function assessment

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SUMMARY Urine samples from neonates admitted to a special care baby unit were analysed to establish ranges for urinary retinol binding protein, albumin, and total protein concentrations in healthy term and preterm infants and to investigate changes seen in disease states. Urinary excretion of retinol binding protein was greater in preterm infants and was increased in sick infants. This was greater than would be predicted from changes in creatinine excretion with gestational or postconceptional age. Urinary retinol binding protein appeared more sensitive to illness than did urinary albumin or total protein.

The measurement of renal function in neonates is not easy: it is difficult to obtain timed urine specimens and blood sampling requires skill. Biochemical techniques that could be performed on untimed urine samples to predict changes in renal function would therefore be useful. In addition, current sensitive and specific techniques to measure individual proteins can now be used to differentiate between glomerular and tubular dysfunction.

The increased excretion of albumin in urine, before dipstick methods or routine laboratory total protein methods showed abnormal results, was used to predict glomerular damage and later renal failure in diabetic patients.[1 2] Other studies have shown that plasma proteins with molecular weights less than 30 000 daltons (for example, retinol binding protein and β_2 microglobulin) are freely filtered at the glomerulus and reabsorbed at the proximal renal tubule.[3] Their reabsorption is normally quantitative and therefore increased excretion of these proteins may indicate renal tubular damage. β_2 microglobulin has been used in the past to assess renal tubular function and has been measured in infants born through meconium stained liquor.[4] There are no reports of urinary retinol binding protein excretion in healthy and sick neonates.

Retinol binding protein is now known to be more stable in normal acid urine than is β_2 microglobulin and should therefore be a preferred indicator of tubular function.[5] Measurement of albumin concentrations should give a measure of glomerular function.

The degree of renal function in utero and in the neonatal period is affected by the gestational age of the infant and by factors such as infection and concomitant treatment with antibiotics. Renal function should therefore be monitored during the treatment of infection to minimise adverse affects on renal function and if possible to give early warning of impending renal damage. Such investigations are complicated by changes in renal function with degree of prematurity.

In this study the urinary excretion of retinol binding protein, albumin, and total protein were measured to establish their normal ranges in healthy term and preterm babies and to investigate the effects of prematurity, illness, and antibiotic drug treatment on their excretion.

Patients and methods

Samples were collected from 221 babies admitted to the special care baby unit between October 1985 and June 1986. Babies were excluded from the study if they were known to have specific renal pathology, for example a congenital malformation or renal venous thrombosis. Information on gestation, birth weight, requirement for increased inspired oxygen concentration, need for ventilation, presence of jaundice requiring phototherapy, administration of antibiotics, and feeding (milk, intravenous glucose, or total parenteral nutrition) was collected on all babies. This information was used to divide the subjects into three groups: 'well', 'special care' and 'intensive care'. The well babies were not given antibiotics, did not need ventilation, and were fed on milk. The special care babies received intravenous

dextrose with or without parenteral antibiotics. The intensive care babies required antibiotics, total parenteral nutrition, and some also required ventilation.

The antibiotics used were cefotaxime alone (48 babies) or netilmicin and benzylpenicillin in combination (42 babies). Eighteen babies received netilmicin alone and 21 received benzylpenicillin and cefotaxime together. It was hoped that the study would show up any differences in renal toxicity between these antibiotic regimes.

The babies who were given antibiotics had all had an infection screen performed. The results of this, which included blood culture, urine culture, surface swabs, erythrocyte sedimentation rate, C reactive protein, and white cell count with differential count, were used to decide whether sepsis was present.[6]

Untimed urine specimens were collected in a bag applied to the perineum and stored frozen at −20°C until their analysis. Specimens contaminated with faeces were discarded. Most specimens (60·9%) were collected during the first 2 days of life, with a few (12·9%) being collected from babies over 1 week of age. Only the results from the first sample from each baby were included in the statistical analysis.

Blood samples were only taken for assessment of renal function where the clinical condition of the baby merited it; therefore the results for plasma urea and creatinine are incomplete. Plasma urea and creatinine were measured by routine biochemical techniques. Urinary retinol binding protein was measured by radioimmunoassay.[7] The coefficient of variations of the method were approximately 6% for quality control samples with concentrations between 20 and 500 μg/l.

Table 1 *Gestation and health of subjects*

	Gestation (weeks)			*Total*
	<30	*30–36*	*>36 (term)*	
Group:				
Well	0	31	25	56
Special care	9	59	58	126
Intensive care	22	13	4	39
Total	31	103	87	221

Table 2 *Plasma urea and plasma and urinary creatinine. Results for plasma urea and plasma creatinine are means (numbers of babies) and differences between means with 95% confidence intervals. Results for urinary creatinine are geometric means (numbers of babies) and ratios with 95% confidence intervals*

	Gestation (weeks)		
	<30	*30–36*	*>36*
Plasma urea (mmol/l):			
Well	—	3·32 (17)	4·05 (14)
Special care	4·57 (9)	3·97 (46)	3·20 (44)
Intensive care	4·76 (21)	3·38 (13)	6·97 (4)
Special care−well	—	0·65 (−0·32 to 1·62)	−0·85 (−2·24 to 0·53)
Intensive care−well	—	0·07 (−1·26 to 1·39)	2·92 (−0·8 to 6·60)
Intensive care−special care	0·19 (−2·51 to 2·12)	−0·58 (−1·82 to 0·66)	3·78 (0·0 to 7·58)
Plasma creatinine (μmol/l):			
Well	—	83·2 (4)	58·5 (6)
Special care	88·1 (9)	76·8 (34)	70·1 (22)
Intensive care	86·2 (15)	70·3 (7)	134·0 (2)
Special care−well	—	−6·43 (−29·3 to 16·5)	11·6 (−11·8 to 35·0)
Intensive care−well	—	−12·9 (−34·9 to 8·9)	75·5 (−520 to 670·8)
Intensive care−special care	−1·91 (−19·7 to 23·5)	−6·54 (−18·7 to 5·6)	63·9 (−524 to 651·6)
Urinary creatinine (mmol/l):			
Well	—	1·61 (31)	2·67 (25)
Special care	1·07 (9)	1·61 (59)	2·32 (58)
Intensive care	1·02 (22)	1·52 (13)	5·33 (4)
Special care−well	—	1·00 (0·72 to 1·39)	0·87 (0·54 to 1·39)
Intensive care−well	—	0·95 (0·58 to 1·54)	2·00 (0·69 to 5·76)
Intensive care−special care	0·95 (0·55 to 1·65)	0·94 (0·60 to 1·48)	2·30 (0·83 to 6·35)

Urinary total protein was measured by turbidimetry using benzethonium chloride precipitation.[8] Urine albumin was measured by immunoturbidimetry on an LKB 8086 reaction rate analyser.[9] The coefficient of variation of the method was 10% at 30 mg/l. Urinary creatinine was measured by a pseudo kinetic modification of the Jaffé method.

Data were analysed using the Statistical Package for the Social Sciences x (release 2.2) and Minitab (revision 5.1.3). Results of urinary proteins were expressed as ratios to the urinary creatinine concentration to correct for differences in urine flow rate. These urinary protein:creatinine ratios exhibited a skewed distribution and were $\log_{10}$ transformed for analysis. No attempt was made to relate creatinine excretion to body surface area. Significance was tested using analysis of variance, differences between groups were tested using the least significant difference test at the 1% level. The effects of combinations of variables were investigated using multiple regression analysis.

Results

Two of the 129 babies given antibiotics had sepsis as shown by positive blood cultures; a further 43 were probably infected as shown by other indicators of infection such as a raised C reactive protein, white cell count, or erythrocyte sedimentation rate.

The distribution of gestational age at birth is shown in table 1. None of the infants of less than 30 weeks' gestation at birth were considered healthy; this is to be expected as over 50% of babies of this gestational age would develop respiratory distress syndrome and require supportive treatment.[10] Tables 2 and 3 summarise the plasma and urine results. Urinary creatinine results were within the range described by Sertel and Scopes, who studied a population of well, term babies.[1]

In the three gestational age groups there were trends towards higher levels of albumin:creatinine, retinol binding protein:creatinine, and total protein: creatinine excretion ratios with increasing degrees of

Table 3 *Urinary albumin, retinol binding protein, and total protein: creatinine ratios. Results are geometric means (numbers of babies) and ratios with 95% confidence intervals*

	Gestation (weeks)					
	<30		*30–36*		*>36*	
Urinary albumin:creatinine (mg:mmol):						
Well	—		21·3	(31)	11·9	(25)
Special care	26·2	(9)	18·4	(59)	13·8	(57)
Intensive care	36·4	(22)	21·1	(13)	58·7	(4)
Special care–well	—		0·86	(0·82 to 0·90)	1·17	(0·70 to 1·96)
Intensive care–well	—		0·99	(0·92 to 1·06)	4·97*	(1·55 to 15·9)
Intensive care–special care	1·39	(1·09 to 1·76)	1·15	(1·08 to 1·23)	4·26	(1·40 to 13·0)
Urinary retinol binding protein:creatinine (μg:mmol)						
Well	—		251·2	(31)	39·4	(25)
Special care	1845·0	(9)	365·9	(59)	123·3	(55)
Intensive care	2454·7	(21)	727·8	(13)	1849·2	(4)
Special care–well	—		1·46	(0·68 to 3·14)	3·13*	(1·38 to 7·07)
Intensive care–well	—		2·90	(0·92 to 9·10)	46·94*	(7·60 to 289·8)
Intensive care–special care	1·33	(0·87 to 2·05)	1·99	(0·69 to 5·75)	15·00*	(6·06 to 37·14)
Urinary total protein:creatinine (mg:mmol):						
Well	—		200·9	(31)	108·3	(25)
Special care	312·0	(9)	193·9	(58)	137·0	(56)
Intensive care	315·6	(22)	204·3	(13)	368·1	(3)
Special care–well	—		0·97	(0·74 to 1·26)	1·27	(0·98 to 1·63)
Intensive care–well	—		1·02	(0·68 to 1·52)	3·40*	(1·79 to 6·47)
Intensive care–special care	1·01	(0·89 to 1·15)	1·05	(0·87 to 1·27)	2·69*	(1·86 to 3·89)

illness (see table 3); these changes were significant in the term (greater than 36 weeks) babies. The differences between the well, special care, and intensive care groups that were significant are shown in table 3. Only the well and intensive care groups could be distinguished for urinary albumin:creatinine ratios, whereas all groups were different for the retinol binding protein:creatinine ratio. The well and special care groups were different from the intensive care group but were not different from each other for the total protein:creatinine ratio. The increases in the term babies were larger than those in the 30–36 week gestation group.

Multiple regression analysis did not show any significant effects due to antibiotic administration or to the choice of antibiotic used.

The association between urinary retinol binding protein and urinary albumin:creatinine ratios for all babies gave a correlation coefficient of 0·58 ($p<0·001$). This only accounts for a 35% agreement between these two measurements, however, and is consistent with their indicating different aspects of renal function.

It can be seen from table 3 that the well, term babies had lower values for urinary retinol binding protein:creatinine, albumin:creatinine, and total protein:creatinine ratios when compared with the well babies of the 30–36 week gestation group. These differences were significant for the retinol binding protein:creatinine and total protein:creatinine ratios ($p<0·001$).

Table 4 summarises the results for well babies of 30–36 weeks and over 36 weeks' gestation. It can be seen that the well babies of over 36 weeks' gestation had lower values for urinary retinol binding protein: creatinine, albumin:creatinine and total protein: creatinine ratios when compared with the well babies of the 30–36 week gestation group. These differences were significant for the urinary retinol binding protein:creatinine and total protein: creatinine ratios ($p<0·001$).

Table 4 *Normal ranges for well babies. Results are geometric mean and mean (1·96 SD)*

	Gestation (weeks)	
	30–36	*>36*
Urinary albumin: creatinine (mg:mmol)	21·3, 1·32 (343·8)	11·9, 1·78 (78·63)
Urinary retinol binding protein: creatinine (μg:mmol)	251·2, 0·50 (19828·0)	39·4, 2·92 (533·2)
Urinary total protein: creatinine (mg:mmol)	200·9, 41·3 (976·8)	108·3, 55·0 (213·2)

Discussion

Retinol binding protein is present in the circulation of the fetus either free or bound to prealbumin. In the preterm infant the plasma retinol binding protein is mainly from maternal sources. It was reported by Ismadi and Olsen that the human fetus does not produce significant amounts of retinol binding protein until about 34 weeks' gestation.[12] Howells *et al* showed that plasma retinol binding protein did not correlate with either gestational age or birth weight.[13] Legge did not find any retinol binding protein in amniotic fluid[14]; however, the assay used was less sensitive than the radioimmunoassay used in this study.

In the well babies (table 4) we have shown a sixfold decrease in urinary retinol binding protein: creatinine ratios and a twofold decrease in urinary total protein:creatinine ratios with the two gestational age groups at birth ($p<0·0001$). The urinary albumin:creatinine ratios showed a similar trend but at a lower level ($p<0·05$). These results provide tentative reference ranges for the excretion of these proteins in term and preterm neonates.

The changes in renal function with increasing gestation as measured by glomerular filtration rate and plasma creatinine have been shown previously.[15,16] Both these methods require blood sampling to be carried out.

The decrease in the urinary retinol binding protein: creatinine that we have shown may indicate more efficient proximal tubular function as gestation progresses, which would agree with the more efficient glomerular filtration shown by others.[11] The relatively minor changes in the urinary albumin: creatinine ratio with gestation may indicate that this is a less sensitive measure of renal function than tests of proximal tubular function in early life. A protein:creatinine ratio would be expected to decrease with increasing gestational age and skeletal muscle mass (table 2). However, the increase in urinary creatinine appears insufficient to account for the significant decrease in the retinol binding protein:creatinine ratio in the well 30–36 and >36 weeks groups.

Having described reference ranges for urinary retinol binding protein, albumin, and total protein: creatinine ratios, it is now possible to investigate those differences that occur in illness. In this study the sick babies mainly has respiratory illness (either idiopathic respiratory distress syndrome or congenital pneumonia) or generalised septicaemia, although bacteriological confirmation of sepsis was

obtained in only two babies. Unsuspected renal tubular dysfunction has been described by Cole *et al* in term babies born through meconium stained liquor[4]; this was detected by measuring β_2 microglobulin in the urine. Increased urinary excretion of small molecular weight proteins may result from competition for reabsorptive sites in the proximal tubule. Such competition may occur from the presence of free branched chain amino acids as are present in intravenous feeding regimes. However, other studies have not shown branched chain amino aciduria in infants on intravenous feeds (V Walker, personal communication).

Plasma creatinine has been used as a measure of renal dysfunction in neonates but, in adults, there is no significant rise until at least 50% of the glomeruli are damaged. It would be useful in the management of fluid and electrolyte balance in neonates to be able to detect renal damage before this degree of loss of function has occurred.

The concentrations of plasma creatinine in all three gestational age groups did not change significantly with severity of illness (table 2), and all lay within the reference ranges described by Rudd *et al*.[17] Thus the measurement of plasma creatinine alone would not have detected any renal dysfunction in the babies studied.

The changes in urinary proteins with increasing severity of illness are most pronounced in the measurement of the retinol binding protein: creatinine ratio in each gestational age group. This suggests that proximal tubular function may be disrupted more than glomerular function in neonatal disease. The increase in the retinol binding protein: creatinine ratio with illness is most noticeable in the over 36 week gestation group—this may be because the well babies of this gestation have relatively mature kidneys, and illness requiring special care is of a more severe nature than in preterm infants who have less general reserve and therefore require more care for less severe illness.

The number of babies in the sick term group was too small (see table 1) to allow the drawing of definite conclusions from the results.

The results in the 30–36 week gestation group show no changes in ratios for urinary albumin: creatinine or total protein:creatinine with varying severity of illness. This probably reflects the overall immaturity of the kidney even in well babies. The urinary retinol binding protein:creatinine ratio does show a trend with severity of illness, but the ranges are such that significance is not reached. However, these results suggest that the urinary retinol binding protein:creatinine ratio may be the most useful for detecting early renal dysfunction in neonates.

In the babies of less than 30 weeks' gestation it is difficult to evaluate the results as there were no normals to compare with the sick babies. However, the results for all variables are generally higher than for more mature babies, again suggesting renal immaturity, especially of the proximal tubule.

This preliminary study indicates that measurement of specific urine proteins may provide sensitive indicators of changes in renal function in neonates either from differences in gestational age or from sickness. Changes in glomerular function appear less dramatic than changes in renal tubular function. The study did not detect differences in renal function related to antibiotic administration but numbers were small. The sensitivity of these non-invasive biochemical markers indicate they may be of use in further studies of renal function in the neonatal period.

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