Amniotic fluid *N*-acetyl- β -D-glucosaminidase activity and renal abnormalities

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Abstract
Amniotic fluid *N*-acetyl- β -D-glucosaminidase (NAG) activity was measured in 21 normal pregnancies to determine reference values, and in 10 pregnancies with fetuses who had prenatally recognised renal and urinary tract malformations. The normal values obtained at 17–19 and 28–31 gestational weeks did not differ, and an activity of 105 units NAG/mmol creatinine was the upper limit of the normal range. Raised NAG activities were found in four fetuses with oligohydramnios and severe bilateral renal disease, and also in two infants with a normal amount of amniotic fluid, only unilateral renal lesions, and a normal renal function after birth. Three of four cases with normal NAG activities had malformations without kidney damage, but one baby with prune belly syndrome was subsequently found to have dysplastic kidneys and renal failure. Our preliminary results suggest that a high level of NAG activity in the amniotic fluid indicates unilateral or bilateral kidney damage but is not proof of intrauterine renal failure.

The exact evaluation of fetal renal function and the demonstration of intrauterine kidney damage are two unsolved problems in the management of children with prenatal diagnosis of renal or urinary tract malformations. The extent of renal functional impairment seems to be best defined by the presence of oligohydramnios, as amniotic fluid is predominantly a result of fetal urine production.¹ Determination of sodium and osmolality of the fetal urine are widely accepted as the most reliable additional parameters.^{2,3} Analysis of fetal urine necessitates the puncture of the fetal bladder or the renal pelvis. Amniocentesis is less difficult and dangerous but no reliable indicator of fetal renal disease has so far been found in the amniotic fluid. In the present study we measured *N*-acetyl- β -D-glucosaminidase (NAG) activity in amniotic fluid in a control group undergoing amniocentesis, mostly for genetic counselling,

to determine the reference values. These results were compared with NAG activities in 10 cases with prenatally recognised urinary tract malformations to determine the value of NAG activity for the evaluation of fetal renal function and for the detection of intrauterine kidney damage.

Patients and methods
Amniocentesis was performed as a routine procedure in 21 pregnancies for genetic counselling or monitoring of suspected rhesus incompatibility. These patients served as the control group, and 15 of them had sampling between 17 and 19 weeks of gestation, and six between 28 and 31 weeks of gestation (table 1). Renal malformations were excluded in all fetuses by sonography.

The activity of NAG in the amniotic fluid was measured photometrically (Boehringer, Mannheim, Germany) with cresol-sulfonphthalein-glucosaminide as substrate, as previously described.⁴ Creatinine was measured by autoanalyser using the kinetic Jaffé reaction. The NAG values were expressed as units per litre (U/l) and standardised for creatinine (U/mmol creatinine). Values exceeding two standard deviations above the mean were defined as increased.

Analysis of amniotic fluid was also performed in 10 pregnancies with prenatally recognised or suspected renal malformations. The data for these cases are summarised in table 2. Elective abortion was performed in three pregnancies and two infants died shortly after birth. Necropsy or postnatal investigations confirmed the renal malformations in all except case 10, where normal kidneys were found.

Cases 1–4 suffered from the most severe malformations and oligohydramnios was noted in all. Case 5 had prune belly syndrome with immature but normally developed kidneys on histological examination. Cases 6 and 7 are examples of malformations with unilateral loss of renal parenchyma. In contrast, ureteropelvic junction obstruction present in case 8 caused no renal

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Table 1 NAG in amniotic fluid. Results of the control group shown as mean (SD) and range

Gestational age (weeks)	No of cases	NAG		Creatinine (μ mol/l)
		U/mmol creatinine	U/l	
17–19	15	73·81 (12·39) 45·20–91·10	4·59 (0·81) 2·80–5·95	62·33 (5·96) 53–70
28–31	6	59·97 (21·43) 31·90–81·90	5·38 (1·71) 2·55–7·00	91·50 (14·21) 80–115
Total	21	69·86 (16·23) 31·90–91·10	4·81 (1·15) 2·55–7·00	70·66 (16·05) 53–115

Table 2 Clinical data and NAG activity of 10 cases with prenatally suspected renal disease

Case No	Gestational age (weeks)	NAG		Prenatal findings	Outcome	Definitive diagnosis
		U/mmol creatinine	U/l			
1*	24	337·10†	23·20†	Polycystic kidneys	Abortion	Infantile polycystic kidney disease
2*	18	181·70†	10·90†	Bilateral hydronephrosis	Abortion	Posterior urethral valve, dysplastic kidneys
3*	32	151·40†	10·60†	Small, echodense kidneys	Died at 2 days of age	Dysplastic kidneys, pulmonary hypoplasia
4*	37	305·60†	27·50†	Multicystic right kidney	Died at 26 days of age	Multicystic right and dysplastic left kidney, trisomy 21
5	15	80·00	4·00	Enlarged bladder	Abortion	Prune belly syndrome, normal kidneys
6	31	116·25†	9·30†	Bilateral hydronephrosis and hydroureter	Alive	Bilateral vesicoureteric reflux grade 5, dysplastic left kidney
7	30	205·00†	12·30†	Left hydronephrosis	Alive	Multicystic-dysplastic left kidney
8	27	46·00	4·60	Left hydronephrosis	Alive	Obstruction of left ureteropelvic junction, normal kidneys
9	20	79·30	5·55	Fetal ascites, enlarged bladder	Alive, renal failure	Prune belly syndrome, dysplastic kidneys
10	33	65·00	5·20	Large, echodense kidneys	Alive	Normal kidneys

*Oligohydramnios.

†Increased NAG values.

lesion. Case 9 had prune belly syndrome, with fetal ascites, a patent urachus, and normally developed kidneys without hydronephrosis at the time of investigation. Eight weeks later, oligohydramnios and bilateral hydronephrosis were noted, but no fetal ascites. Investigations after premature delivery at 32 gestational weeks showed dysplastic kidneys which led to renal failure, and severe urethral stenosis.

Fully informed consent of the parents was obtained for all investigations. Student's *t* test was used for statistical analysis.

Results

The results of the control group are listed in table 1. NAG values were not statistically different in the two age groups although the values obtained between 28 and 31 gestational weeks were slightly higher if expressed as units per litre. In contrast, creatinine values were significantly higher in the later gestational weeks ($p < 0.01$). According to these findings, NAG values of 105 U/mmol creatinine were defined as the upper limit of the normal range.

NAG activity was raised in all four cases with oligohydramnios (cases 1–4) and severe bilateral renal disease. Two infants with unilateral renal lesions on postnatal investigation (cases 6, 7) also had raised NAG values, while three with normal kidneys had normal NAG activities despite malformations in two of them (cases 5, 8). Yet there was one infant (case 9) with prune belly syndrome who had a normal NAG activity but was found after birth to have badly damaged dysplastic kidneys and renal failure.

Discussion

NAG is a lysosomal enzyme which is present in high concentrations in renal tubular cells. Normally only small amounts are excreted into the urine. The excretion rate is not affected by serum concentrations as NAG is not filtered by the glomeruli. Raised urinary NAG values are found in various renal diseases. They reflect the activity of the disease and indicate renal damage.^{5,6} Amniotic fluid is mostly composed of fetal urine and therefore NAG is found as a

normal constituent. Recently Agosti *et al* found constant NAG activities in the amniotic fluid between 16 and 19 weeks of gestation.⁷ Our data confirm these findings and in addition suggest similar NAG values in later stages of pregnancy. The slightly higher values of NAG if expressed as U/l and of creatinine obtained between 28 and 31 weeks of gestation could reflect the fetal growth and the greater mass of the fetal kidneys, but this difference is excluded if NAG is standardised for creatinine.

NAG isoenzyme studies showed that amniotic fluid as well as the urine of neonates exclusively contain the B-isoenzyme.⁷ This finding indicates that fetal urine production is actually the main source of NAG in amniotic fluid. Amniotic cells express lysosomal enzymes, including NAG activity. No conclusive information is yet available on how much NAG activity may originate from these cells, especially in a pathological state.

Despite these considerations, our results seem to indicate that an increased NAG activity in the amniotic fluid is associated with intrauterine renal disease. Increased NAG activities were found not only in the most severe cases with bilateral renal disease and oligohydramnios (cases 1–4) but also in two cases with a normal amount of amniotic fluid, only unilateral loss of renal parenchyma, and a normal renal function after birth being provided by the contralateral kidney (cases 6, 7). One of these had a non-functioning unilateral multicystic kidney. Increased NAG activity could be interpreted as a severe ureteropelvic junction obstruction causing the complete destruction of the kidney. These two cases are particularly important. They show that an increased NAG activity is no proof of intrauterine renal failure. Rather it seems to indicate that kidney damage is present or developing. Therefore this test could be helpful in distinguishing fetuses with malformations leading to intrauterine loss of renal parenchyma from fetuses with malformations which are, at least at the time of investigation, not harmful for the kidneys and the renal function. Case 9 had a normal NAG activity despite severe renal disease after birth. Our speculation is, that at the time of amniocentesis

the patent urachus served as a urinary drainage enabling a normal renal development, and that the loss of renal parenchyma took place after its closure.

Our study was performed in a small number of cases and the results are preliminary. However, further investigations seem to be justified as this test could be helpful in clarifying some aspects of intrauterine renal disease.

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