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URINARY N-ACETYL-β-D-GLUCOSAMINIDASE ACTIVITY IN HEALTHY, POLYCYTHEMIC AND HYPOXIC NEONATES

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The authors investigated the urinary N-acetyl-β-D-glucosaminidase (NAG) activity in the case of 101 normal healthy and 20 polycythemic newborns and prematures, and 50 prematures suffering from hypoxia on the 1st, 2nd, 4th, 14th, and 28th day after birth. The obtained activities were referred to the creatinine concentrations of the urine samples and given as NAG index. There were no significant differences in the NAG indices either between fullterm and preterm babies or between appropriate for gestational age (AGA) and small for gestational age (SGA) neonates of the normal group. The NAG indices on the first day of life were significantly higher in the case of polycythemic newborns in comparison with the normal group ($p < 0.01$). On the 14th day, after the partial plasma exchange, the NAG indices returned to the normal range. The premature babies suffering from IRDS received an average 10.1 days oxygen supplementation. Their NAG indices were significantly ($p < 0.01$) higher on the 1st, 2nd, 4th days than those of the healthy prematures of the normal group and decreased considerably up to the 14th day. Finally the NAG indices reached the normal value on the 28th day. These results support the assumption that the urinary NAG index is a suitable indicator of the renal tubular damage during the newborn period.

INTRODUCTION

The nephrotoxic drugs widely applied in intensive neonatal treatment, the temporary hypoxic periods and the pathological oscillation of the oxygentension may cause tubular damage, which has been studied very intensively /6,14,18,21/. The degree of the damage can be estimated on the basis of the

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characteristic increase in either the total urinary protein content or some typical enzyme activities of the urine, e.g. N-acetyl-β-D-glucosaminidase (EC.3.2.1.30).

The different NAG isoenzymes /29,31/, which can also be found in high concentration in the proximal tubule cells of the kidney, are lysosomal hydrolases with a molecular weight of about 140 000 daltons.

Physiologically they play an important role in the catabolism of both glycoproteins and mucopolysaccharides. The NAG satisfies the Gonick's criteria for selecting enzymes potentially useful in the detection of different renal diseases /7/.

In connection with adult patients recently a lot of publications have been reported on the successful diagnostic applications of this enzyme /10,12,14/ e.g. lead poisoning /19/, Allopurinol treatment /20/, for the indication of rejection reaction after renal transplantation /13/, for the diagnosis of renal complication in diabetes mellitus /23,33/, for the diagnosis of renal changes during pregnancy /26/, and for the diagnosis of other renal diseases /11,31/. However, relatively little data can be found in the literature in relation to the application of this method during neonatal period and in childhood /8,22,27,28,30,31,32/. Only one research group reported on the normal value for healthy neonates on the basis of a few cases /15/. Therefore, our main goals were the investigation of the applicability of this method i.) for measuring the degree of the damage and ii.) for the diagnosis of hypoxic tubulopathy, caused by polycythemia and hypoxia, iii.) for the detection of the temporal development of the process.

PATIENTS AND METHODS

Between November 1st, 1988 and May 1st, 1989 101 normal and 20 polycythemic patients were investigated. The details of these patients are summarized in Table I. The normal neonates did not need oxygen supplementation, did not have urinary infection and were not given antibiotics.

TABLE I

Details of te normal and polycythemic patients

		Number of patients[a] male	female	Gestational age[b] weeks (mean ± SD)	Birth weight[b] gram
Full-term	AGA	30 (0)	20 (0)	38.9 ± 1.3	3353 ± 417
	SGA	12 (3)	10 (0)	38.6 ± 1.1	2210 ± 233
Preterm	AGA	9 (5)	17 (9)	35.4 ± 0.9	2280 ± 125
	SGA	2 (2)	1 (1)	35.0 ± 1.1	1740 ± 80

a: the number of polycythemic patients is indicated in parenthesis

b: there were no significant differences between healthy and polycythemic patients in gestational age and birth weight

The hematocrit (Htc) was determined from venous blood within 2 hours or between 12 - 24 hours after birth /5,17/. In the case of any following pathological alterations: tachypnoe, hypoglycaemia, hypocalcaemia, thrombocytopenia and if the Htc was greater than 65 per cent, partial exchange transfusion was performed without delay, with 5 % human albumin. If the above mentioned pathological alterations could not be detected, the partial plasma exchange was only made if the Htc was greater than 70 per cent.

The urine specimens were collected on the days 1st, 4th, 14th, for twelve-hour-periods, using urine collection bags. These 101 patients were divided into subgroups according to sex, gestational age, the degree of retardation if any, and polycythemy /Table I/.

During this period 50 preterms with IRDS were investigated in the Neonatal Intensive Care Unit, namely: 25 males, 25 females, gestational age: 31.8 $\pm$ 2.5 weeks (mean $\pm$ SD), birth weight: 1590 $\pm$ 410 gram, one minute Apgar score of: 7.5 $\pm$ 1.5, five minute Apgar score of: 8.3 $\pm$ 1.4. During the observation period half of the patients (15 males, 10 females) died. The neonates required oxygen supplementation for 10.1 $\pm$ 7.0 days on average. The average oxygen concentration (p_cO_2) and oxygen saturation (cap.$SatO_2$) of these patients on the first six days of life can be seen in Table II. Urine samples were obtained on the 1st, 2nd, 4th, 14th, 28th days and were immediately frozen (-20°C) and analysed within two weeks.

For determining the NAG activity the method of Horak et al /9/ was used with slight modifications. The specimens of urine were allowed to thaw at 4°C. After centrifugation (1000 g, 5 min). 1 ml aliquots of the samples were filtered on 8 x 1.5 cm fine mesh Sephadex G - 25 columns (Pharmacia AB, Uppsala, Sweden). The enzyme activity in the eluents was determined by the application of chromogenic p-nitrophenyl-β-D-glucosaminid substrate /16/. The reaction was carried out in 0.1 M sodium citrate buffer, pH 4.4 at 37°C and quenched with 0.2 M sodium borate buffer, pH 10.0. The amount of the liberated p-nitrophenolate was determined spectrophotometrically at 400 nm with a SPECORD M -40 device, by the use of calibration curves. In every case substrate blank solution was used. The enzyme activity was expressed in terms of the hydrolysed p-nitrophenyl-β-D-glucoside (μmol/min/l).

The urine creatinine concentration was determined by Jaffe' method /2/ with Centrifichem autoanalyser.

Statistical analysis was made on the basis of the Student's t-test /3/.

RESULTS

The NAG indices of the normal patients on the 1st, 4th, 14th days after birth according to sex, gestational age (fullterm - preterm), the degree of retardation (AGA - SGA) are shown in Fig. 1. The increase of the NAG index observed in the females

TABLE II

Average capillary oxygen tension (p_cO_2) and oxygen saturation (cap. $SatO_2$) in preterm infants with idiopathic respiratory distress syndrome on the first six days of life.

p_cO_2 (Hgmm) (mean ± SD)	53 ± 19	57 ± 22	62 ± 21	58 ± 22	54 ± 20	61 ± 24
cap.$SatO_2$ (%) (mean ± SD)	77 ± 16	82 ± 15	86 ± 11	80 ± 18	79 ± 17	82 ± 16
Days	1	2	3	4	5	6

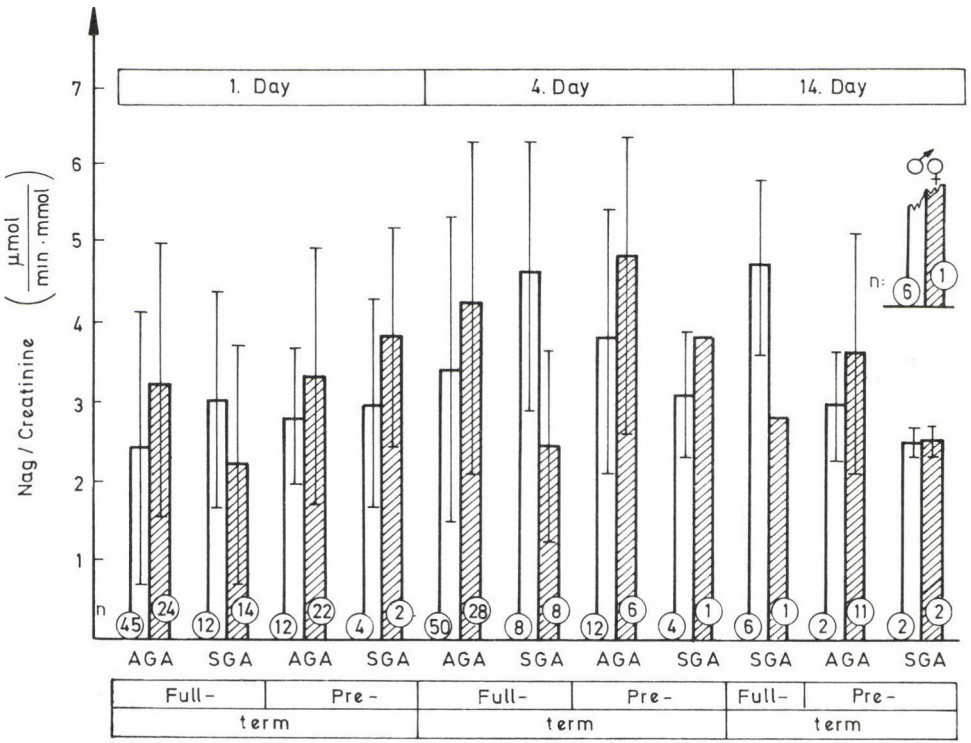

Fig. 1. Urinary NAG index in healthy full-term and preterm infants (mean ± SD).

was only significant in the fullterm AGA group in the first day of life ($p < 0.05$). Moreover, there were no significant differences between the fullterm and preterm, AGA and SGA groups compared on any day of observation.

The NAG indices of the polycythemic neonates can be seen in Fig. 2. It is noteworthy, that the NAG indices of this group were significantly higher than the normal values in all sub-groups on the day of life ($p < 0.01$). On the 4th day considerable decrease of the NAG indices could be observed except in the preterm AGA boys, but these values still exceeded the normal ones ($p < 0.05$). On the 14th day the NAG index went back to the normal range. Simultaneously, the differences in the NAG index between the males and females both of the preterm AGA and preterm SGA sub-groups were negligeable on any day during the investigation.

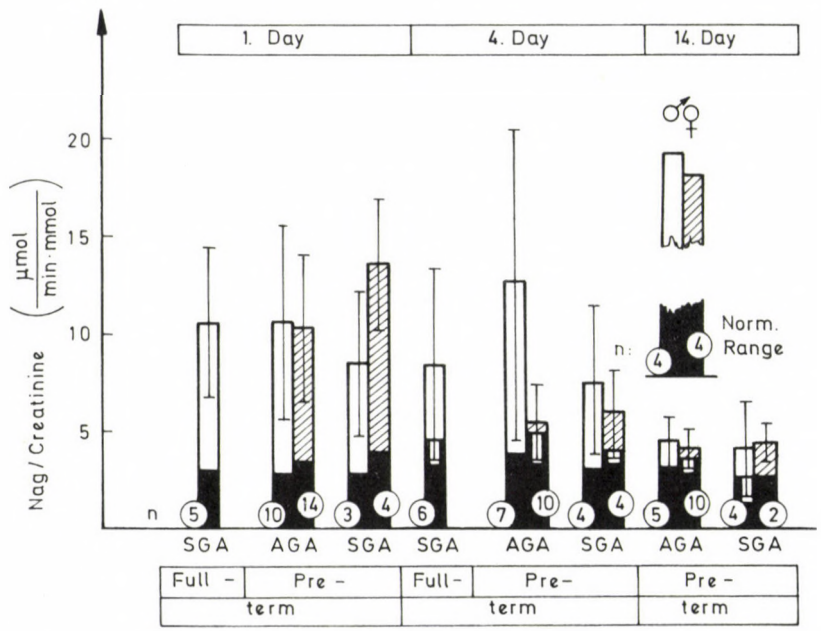

Fig. 2. Urinary NAG index in full-term and preterm infants with polycythemia (mean ± SD).

Fig. 3 indicates that the NAG indices of the preterms with IRDS who participated in intensive therapy were significantly higher on the 1st, 2,nd, 4th, days than the normal values ($p < 0.01$). The NAG index decreased up to the 14th day and became normal on the 28th day. On the 1st, 2nd, 4th, 14th days there was no significant difference between the NAG indices of males and females. On the other hand, on the 14th, and the 28th days the NAG indices of males exceeded the corresponding values of females which might be attributed to the prolonged reparation of males.

DISCUSSION

The urinary proteins and enzymes (alanin-aminopeptidase, β_2-microglobulin, lysozym) are extensively applied nowadays for the detection of the nephrotoxic effects of different drugs (e.g. aminoglycosides, indomethacin hypoxia, etc. The measuring of the β_2-microglobulin (β_2M) of the urine has become an especially widely used method in the medical routine /1,4,25/ and the possible diagnostic application of the urinary NAG determination also seems to be promising. Some publications have reported that the determination of the NAG index, which is a relatively simple procedure, gives useful and valuable information on the condition of the renal tubulus as can be obtained by the application of β_2M tests /8,24/. Moreover, Rajchgot and his co-wokers found that the increase of the NAG index in some cases is an earlier and more sensitive indicator of tubular damage than that of the β_2M level /22/. Although a lot of papers have already been published on the tubulopathic effects of aminoglycosides and other nephrotoxic drugs in childhood and adults /22,27,28,32/, the tubulopathy caused by hypoxia in neonatal period is a much less investigated field.

First of all, we determined the normal ranges of NAG indices for healthy neonates and preterms as a function of sex, gestational age and birth weight (Fig. 1). We assumed that in

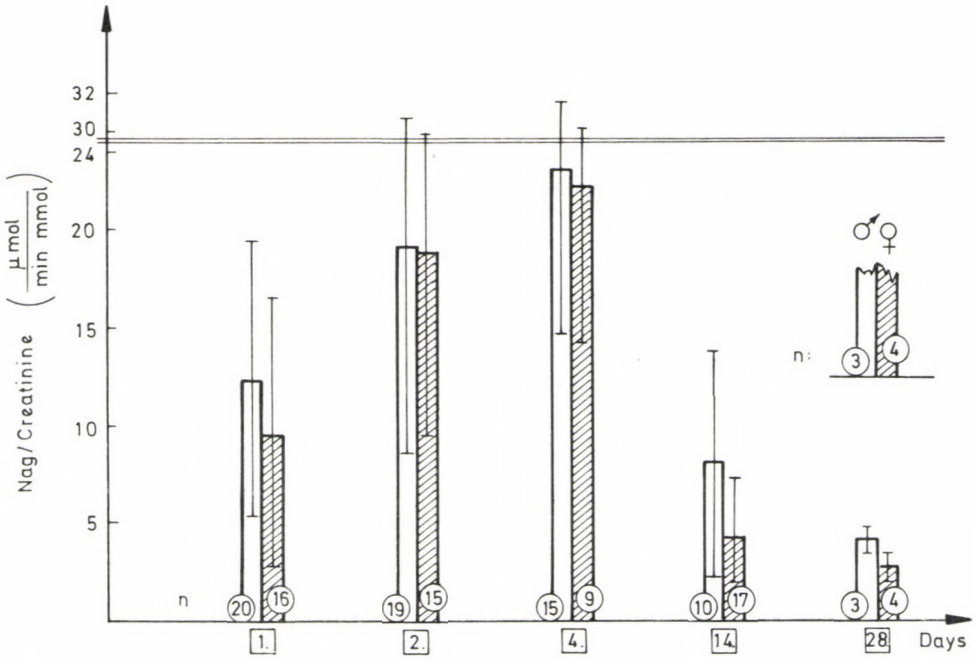

Fig. 3. Urinary NAG index in preterm infants with idiopathic respiratory distress syndrome (mean ± SD).

the case of retarded infants a relative placenta insufficiency led to the observed retardation which might also caused tubular dysfunction in the kidneys. But our investigation did not support this assumption because the NAG indices of the retarded infants did not differ significantly from that of the normal group either in the case of fullterm or preterm babies /Fig.1 /. Therefore, we could conclude that the different processes which led to retardation had no tubulopathic effect detectable with this method. Regarding the dependence of the NAG indices on sex, we found, that they were higher in females, which is in good accordance with previous observations /14/. But this difference between males and females was significant ($p < 0.05$) only in the full-term AGA group on the first day of life. In the case of polycythemic neonates we observed high NAG indices on the 1st day of life, which decreased to the normal value on the 4th day in females and on the 14th day in males, in consequence of partial plasma exchange (Fig. 2). As it can be seen, the NAG indices of the fullterm and preterm polycythemic groups hardly differed from each other (Fig. 2), that is the hypoxic tubular damages were essentiallly independent of the gestational age after the 35th week. The kidney functions of preterms with IRDS, ventillated for a long period of time with a higher concentration of oxygen, can be damaged by hypoxia, pathologic changes in the oxygen tension, as well as by the necessarily applied medicines. Our patients required oxygen supplementation for about 10 days. The continuous increase of the NAG indices on the 1st, 2nd, 4th days persuasively indicate the progress of tubulopathy. On the other hand, the decrease of the NAG index after the 4th day shows that the pathological processes were reversible in these cases, which is a good evidence of the very high regeneration capacity of the kidney (Fig. 3).

On the basis of our results we think that the urinary NAG index determination, a relatively simple and fast procedure, will become a suitable tool for the reliable indication of neonatal hypoxic tubulopathy and for the detection of this process in the near future.

Besides the diagnostic significance, the NAG index may also be applied in planning therapeutic steps for the influence of the renal functions.

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