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Short Communication

Age dependence of serum β-N-acetylhexosaminidase (NAG) activity

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Abstract

Serum N-acetyl-β-D-glucosaminidase (NAG; EC 3.2.1.30) is a hexosaminidase and may be a predictor of vascular injury, e.g., in infant respiratory distress syndrome, pneumonia, broncho-pulmonary dysplasia and necrotizing enterocolitis. To estimate the new diagnostic prospects we have modified our urinary NAG assay. In this sensitive colorimetric micro-assay, VRA-GlcNAc was used as a substrate. In the present study the age dependence of serum NAG activity was investigated in newborn babies, infants (1–24 months), children (2–18 years) and adults (19–80 years). Serum NAG activity was found to be age-dependent; it is higher in early childhood (11–59 U/l) but decreases to a constant value at the age of 1–2 years. After the age of 2 years it is similar to adults' NAG (10–30 U/l). In pediatrics age-matched reference ranges must be taken into consideration.

Keywords: age dependence; infant respiratory distress syndrome (IRDS); reference ranges; serum N-acetyl-β-D-glucosaminidase (NAG); vascular injury; VRA-GlcNAc.

Lysosomal hexosaminidases such as N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) exist in a number of tissues (liver, kidney, spleen and bowel). After tissue damage they might be released into the circulation. In the last decades NAG isoenzymes were used mainly for screening Tay-Sachs disease (TSD) (1, 2). For the previous tests generally plasma or serum is used (2–5), while to detect tubulopathy a urinary sample is used (6–11). Serum NAG may be a possible predictor of vascular injury in diabetes mellitus and hypertension (12, 13). Although hexosaminidase activity varies during postnatal development and depends on feeding, serum NAG has been proposed recently as a predictor of infant respiratory distress syndrome (IRDS), broncho-pulmonary dysplasia (BPD) and pneumonia in premature babies (3). Increase in serum NAG has also been suggested as a marker for the early identification of necrotizing enterocolitis (NEC) (14). NAG activity might be determined by fluorometric methods (15), although colorimetric assays present many advantages (16).

Our aim was to examine the age dependence of serum NAG. Because of the limited volume of serum, the urinary NAG assay (8–10) was modified. This resulted in a simple and sensitive micro-method, which is applicable to different clinical areas. The Kolmogorov-Smirnov test was used to test the normality of distributions, both for NAG enzyme activities and their logarithms. Age dependence was tested using one-way analysis of variance (ANOVA). Dunnet's test was used to compare multiple age groups to the adult group. No significant difference was found in enzyme activities between males and females. NAG activities showed a rather large scatter in newborns, with a distribution not significantly different from Gaussian. In all other age groups NAG activities were log-normally distributed (Kolmogorov-Smirnov test, $p > 0.05$). With ANOVA we found that NAG is age dependent. As indicated by Dunnet's test, NAG is significantly higher below 2 years of age than in adults, and then falls to the adult value. The reference ranges (95% confidence level) are shown in Table 1.

Decreased activity of serum total NAG and isoenzyme A is well known in TSD patients (1, 2). In contrast, the potential value of increased serum NAG in newborns with IRDS, BPD (3) and NEC (14) was confirmed. Very limited data can be found for serum hexosaminidase in newborns and premature babies (4, 14). Our NAG results are similar to the values published earlier (3, 4, 14). In accordance with our results, Lobe et al. (14) showed that NAG activity is independent of sex and is relatively high during the first 3 weeks of life. As they have found higher NAG values after the onset of NEC, or pneumonia, we plan to check whether NAG could be an early detector for these inflammatory diseases. Similarly to our newborns' range, Shattuck et al. described increased NAG activity in premature babies at the 1st–8th weeks of

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Table 1 Reference ranges of serum NAG isoenzyme activities in various age groups.

Age groups		Newborns <1 month, n=22	Infants 1–24 months, n=25	Children 2–18 years, n=24	Adults 19–80 years, n=23
NAG enzyme activity (U/l)	Lower limit	11.0	11.5	10.4	10.0
	Upper limit	48.1	59.3	28.5	30.0

Serum NAG assay (10): 3.3 mmol/l VRA-Glc-NAc substrate (PPR Diagnostics, London, E1W 1AT, UK) was dissolved in citric acid-disodium hydrogen phosphate buffer (0.15 mol/l, pH 4.8) and pre-incubated at 37°C for 5 min. Before the enzyme assay, 25 μl of serum was diluted with 25 μl of the same buffer to decrease the inhibiting effect of urea (17). To 350 μl of substrate solution, 25 μl diluted serum was added and incubated for 30 min at 37°C. The reaction was stopped with 375 μl potassium hydrogen carbonate-dipotassium carbonate buffer (1.2 mol/l; pH 9.8) and the absorbance (A) was read at 505 nm against the reagent blank in a 1 cm cuvette (Humalyser 2000, Human, Germany). Serum samples were stored at −20°C for 1–4 weeks. NAG activity was calculated directly via specific molar absorbance: NAG activity (U/l) = A(505)×28.5×2.

life (4). Recently it was suggested that NAG might be a marker of oxidative stress, e.g., in diabetic microangiopathy, as it correlates with plasma malondialdehyde in diabetes mellitus type 2 (18). Although serum NAG is not a specific marker for vascular diseases, it may contribute to the early diagnosis of the diseases mentioned above.

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