

BRIEF REPORT

Isoenzyme A and Urinary *N*-Acetyl- β -D-Glucosaminidase Activity in Normal Pregnancy

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Abstract

Objectives: Urinary *N*-acetyl- β -D-glucosaminidase (NAG) activity has been found to increase during normal uncomplicated pregnancy and such behavior could limit the diagnostic value of this enzyme for detection of subclinical tubular injury. The aim of this study was to evaluate urinary NAG activity and isoenzyme A in normal pregnant women at 30th week of pregnancy and in healthy women, to discriminate between physiological and lesional enzymuria. **Design and methods:** Enzyme activities in first morning fasting urine samples from 20 nonpregnant control and 20 normal pregnant women at 30th gestational week were evaluated by fluorometric methods. **Results:** Both total and isoenzyme A activity was significantly higher ($p < 0.01$) in urines of normal pregnant women compared with control urines, whereas ratio between these two parameters was significantly lower ($p < 0.001$). **Conclusions:** The increase of urinary NAG activity during normal uncomplicated pregnancy appears to be characterized by a prevalent increase in isoenzyme A form, a finding associated with functional (not lesional) enzymuria. The fluorometric assays may represent a simple and rapid method to evaluate whether increase in urinary NAG activity represents a renal physiological adaptation during pregnancy.

Keywords: Normal pregnancy, urine, *N*-acetyl- β -D-glucosaminidase, isoenzyme, fluorometric assay

INTRODUCTION

Several urinary enzymes have been proposed as early, noninvasive indicators of kidney injury, namely alanine aminopeptidase (AAP), γ -glutamyl transferase (GGT), acid phosphatase (ACP), matrix metalloproteinase-9, and *N*-acetyl- β -D-glucosaminidase (NAG).^{1–3}

Urinary NAG is the enzyme most extensively studied to evaluate tubular damage in the setting of a wide variety of experimental and clinical conditions, including preeclampsia, eclampsia, and pregnancy-induced hypertension.^{4–6} NAG is a lysosomal enzyme distributed throughout the nephron, but its activity has been found to be two to four times higher in the proximal tubule than in other nephron segments. It is not normally filtered at the glomerulus due to its high molecular weight, so urinary NAG originates from tubular cells; therefore, in the absence of the glomerular damage, NAG will be detected in urine as a result of leakage or exocytosis from damaged or stimulated

tubular cells.^{7,8} Previous findings evidenced that NAG activity in urine of normal pregnant women was significantly higher than in control urines and such an increase in urinary NAG activity during normal uncomplicated pregnancy reaches the maximum value in gestational week 30.^{9–14}

For this reason it has been hypothesized that such a physiological increase in urinary NAG excretion during normal pregnancy could limit the diagnostic value of this enzyme for detection of subclinical tubular injury.¹⁵

However, in human tissues and body fluid NAG exists in two main forms: A (acidic, NAG A) and B (basic, NAG B) and several minor forms (I1, I2, As, and P) which are distinguished according to their different charge characteristics using diethylaminoethyl-cellulose (DEAE-C) chromatography.¹⁶ The subunit composition of A and B isoforms is $\alpha\beta$ and $\beta\beta$, respectively, and the percentage of A isoform is the greatest in normal urine.^{17–19}

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Different and expensive techniques have been carried out for the evaluation of NAG isoenzymes in urine.^{17–23} Noteworthy, all NAG isoenzymes have activity toward 4-methylumbelliferyl-2-acetamido-2-deoxy- β -D-glucopyranoside (MUG) substrate. The presence of α subunit enables NAG A to hydrolyze the GM2 ganglioside and the fluorogenic substrate 4-methylumbelliferyl-2-acetamido-2-deoxy- β -D-glucopyranoside-6-sulfate (MUGS) while NAG B isoform does not hydrolyze the MUGS substrate.²⁴

Previous studies on isoenzymatic profile, evaluated by ion-exchange chromatography, of NAG from various biological sources showed that NAG A activity, evaluated by MUGS, is quantitatively lower than that measured by MUG and is a variable proportion of NAG activity.^{25–27}

The possibility to evaluate the different isoenzymatic profile of NAG has led to the notion of the so-called functional enzymuria mainly linked to preferential urinary excretion of A isoenzyme and the so-called lesional enzymuria with preferential urinary release of the B isoform.^{28,29} However, little is known about the isoenzymatic pattern of urinary NAG during normal uncomplicated pregnancy.

During our detailed and comprehensive studies for several years of the urinary markers of tubular damage we have opportunistically been able to sample urine from women both in nonpregnant status and during normal uncomplicated pregnancy.

The current study reports the isoenzymatic profile of NAG activity, evaluated by fluorometric assays, in urine from normal pregnant women at 30th week of gestation and in urine from healthy nonpregnant women.

MATERIALS AND METHODS

Patients

Twenty normal uncomplicated pregnant women at 30th week of gestation (as calculated from the last menstrual period of the woman concerned) entered the study. Pregnant women with urinary tract diseases, diabetes mellitus, hypertension, liver or pancreas diseases, infections over the previous months, use of nephrotoxic drugs, exposition to heavy metals, smokers, and alcoholics were excluded from the study. *Biochemical markers related to renal parameter such as albuminuria, proteinuria, glomerular filtration rate (GRF), blood urea nitrogen (BUN) assay, as well as urine albumin and standard urinalysis, were in normal range.* Patients who developed pregnancy-related complications were also excluded. The control population consisted of 20 age-matched healthy females.

Urine Samples

“Spot,” mid-stream samples of urine, collected in first morning fasting, were centrifuged at 1500g for 10 min and stored at 4°C. Enzymatic activities were determined

within 24 h from collection and therefore no preservatives were used. An aliquot was used to determine urinary creatinine (Cr).

Chemicals

MUG was purchased from Sigma Chemical Co. (St. Louis, MO, USA). MUGS was obtained from HSC Research Development Corporation (Toronto, Canada).

Enzyme Activity Determination

Total enzymatic activity was determined as previously described,¹⁷ using MUG as substrate in 0.1 mol/L citrate and 0.2 mol/L phosphate buffer (pH 4.5). The assay with MUGS was performed as previously described.³⁰ Fluorescence of the liberated 4-methylumbelliferone was measured on a Perkin Elmer LS-3 fluorometer (PerkinElmer, Branchburg, NJ, USA), with 360 nm excitation and 446 nm emission. The fluorometer was calibrated with 4-methylumbelliferone solution in 0.2 mol/L glycine buffer (pH 10.6). In all measurements, blank substrates and blank samples were used and all determinations of enzyme activity were done in duplicate. One unit of activity (U) is the amount of enzyme required to release 1 nmol/h of 4-methylumbelliferone at 37°C. Urinary enzyme activity is expressed as U/mmol Cr.

Urinary Creatinine Determination

Urinary creatinine was determined by the method described by Henry.³¹

Statistical Analysis

Statistical analysis of the data was carried out using the Analyze-it (Analyze-it Software Ltd., Leeds, UK, demo version). In view of the non-Gaussian distribution of data, a nonparametric test (Mann–Whitney *U* test) was used. Results were considered statistically significant at a *p*-value < 0.05. Values are expressed as median \pm SD.

RESULTS

Table 1 gives the results for NAG and NAG A activities and for NAG/NAG A ratio in two different groups of urine samples. Total NAG activity, determined by MUG substrate, in urine of normal pregnant women was significantly higher ($p < 0.01$) compared with nonpregnant control subjects with a median value about 3.2 times higher than that of controls. Also activity of NAG A isoenzyme, determined by MUGS substrate, was significantly higher ($p < 0.01$) in normal pregnant women than in controls with a median value about 3.6 times higher. On the contrary, the NAG/NAG A ratio was significantly lower ($p < 0.001$) in urine of normal pregnant women with respect to controls.

Table 1. NAG and NAG A activities and NAG/NAG A ratio in urine of nonpregnant and normal pregnant women.

	Nonpregnant women (<i>n</i> = 20)	Pregnant women (<i>n</i> = 20)
NAG activity (U/mmol Cr)	156.2 ± 34.5	488.3 ± 76.9*
NAG A activity (U/mmol Cr)	9.1 ± 1.8	33.1 ± 5.5*
NAG/NAG A ratio	17.1 ± 2.2	15.1 ± 2.6**

Notes: Values are expressed as median ± SD. *n* indicates the number of subjects of each group. A *p*-value less than 0.05 was taken to indicate significance.

p* < 0.01, *p* < 0.001.

DISCUSSION

Increase in excretion of several urinary enzymes has been widely accepted as early markers of tubular damage. Urinary NAG is among the most widely and long-time studied. Kidney undergoes profound changes during normal pregnancy and several renal complications may arise during gestation.^{32,33}

Because physiological changes of same potential urinary tubular biomarkers of kidney damage have been reported also during normal uncomplicated pregnancy, a lot of problems may arise in this setting.

With reference to those enzymes whose activity in normal pregnant women is higher than that reported in nonpregnant women (i.e., NAG and AAP), the importance of establishing a cut-off, by receiver operating characteristic (ROC) curve analysis, has been stressed, and it has been suggested that it could be preferable in this setting to detect those enzymes (i.e., ACP and GGT) whose urinary activity seems not to vary significantly through the course of normal pregnancy.¹⁵

However, further problems may arise. For example, till now, little is known about the behavior of urinary ACP and GGT enzymes during complicated pregnancy. In addition, an increase in GGT activity has been reported during normal pregnancy too.³⁴ The urinary ACP, on the other hand, seems mainly to have an extrarenal origin and does not generally figure among the urinary enzymes reported as potential surrogate markers for acute renal injury.²

Noteworthy, with reference to urinary enzymes undergoing aspecific or physiological increase during normal pregnancy, the evaluation of the urinary isoenzymatic pattern of excreted enzyme could be of great diagnostic help.

Several isoenzymes have been described for urinary NAG whose profile could allow the differentiation of the so-called functional from lesional enzymuria.^{28,29}

To our knowledge, our study is the first report in which the isoenzymatic content of the urinary NAG has been evaluated in women with normal uncomplicated pregnancy.

Normal pregnant women at gestational week 30 and age-matched healthy nonpregnant women were compared because it has been documented that the urinary enzymatic excretion could be influenced by age.³⁵ Urine spots were collected from first fasting urine samples due to a diurnal variation reported for this urinary enzyme.⁴

Urinary NAG was assayed using fluorogenic substrates because their sensitivity is such that the urine can be diluted before testing. Dilution eliminates the effects of possible inhibitors of the enzyme and the need for dialysis before determining enzymatic activity.³⁶ The activity was corrected for urinary creatinine to eliminate variations in enzyme concentration due to urine flow using "spot" samples instead of 24 h samples.³⁷

The NAG activity in urine of normal pregnant women in gestational week 30 was significantly higher than that in control (488.3 ± 76.9 U/mmol Cr vs. 156.2 ± 34.5 U/mmol Cr; ratio 3.2). These results agree with previous reports.²⁶⁻³¹

However, the activity of NAG A isoenzyme in urine of normal pregnant women was also significantly higher compared with urine of controls (33.1 ± 5.5 U/mmol Cr vs. 9.1 ± 1.8 U/mmol Cr; ratio 3.6), while NAG/NAG A ratio (an index of the contribution of NAG B to NAG activity) was significantly lower in urine of normal pregnant women with respect to that of controls.

Taken together, the results of this study show that the increase of urinary NAG activity during normal uncomplicated pregnancy is characterized by a prevalent increase in isoenzyme A form, a finding suggestive of renal functional adaptation to pregnancy and not of kidney damage.^{28,29} The reasons of this increase in total NAG and isoenzyme A activity in urine during normal uncomplicated pregnancy are not clear.

Several changes in anatomy, hemodynamics, and tubular functions are known to occur in kidney during normal uncomplicated pregnancy, together with different hormonal changes from nonpregnant physiological norm, which in turn could influence both the levels and the isoenzyme pattern of urinary NAG.^{32,38,39}

However, the intermediate mechanisms responsible for such a physiological behavior of urinary NAG activity during pregnancy remain to be studied.

Despite the small size of our sample in the present study, we believe that our data are particularly noteworthy and may stimulate further investigations also from other groups of investigators. To our knowledge, there were no data on this type of analysis applied to pregnant women. Our findings suggest that estimation of isoenzyme profile of the increased urinary NAG activity could be a valuable tool to better discriminate physiological enzymuria from lesional enzymuria during pregnancy.

Evaluations are in progress in our laboratory to confirm these preliminary findings in a large number of subjects and to assay the behavior and the evolution of urinary NAG and its isoenzyme profile as a useful index for early diagnosis of tubular damage during complicated pregnancy.

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