Clinical Availability of Urinary N-acetyl-β-D-Glucosaminidase Index in Dogs with Urinary Diseases

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ABSTRACT. Urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) was examined in healthy dogs and dogs with urinary diseases, and its clinical usefulness as an indicator of urinary diseases was discussed. Twenty-eight healthy dogs and 20 dogs with urinary diseases were used. Urinary NAG activity was measured using p-nitrophenyl-N-acetyl-β-D-glucosaminide as substrate, and expressed as units per gram of urinary creatinine (NAG index). Urinary NAG index in urine of healthy dogs was 3.2 ± 2.4 U/g, and NAG index in the dogs with chronic renal failure or lower urinary tract infection accompanied by pyelonephritis was higher than that in healthy dogs. However, the dogs with lower urinary tract infection without pyelonephritis showed normal values of NAG index. Some dogs with diabetic mellitus showed elevated values of NAG index when control of blood sugar was not successful. Increase of NAG index was observed in some dogs with pyometra before increases of BUN and serum creatinine concentration. Therefore, NAG index in urine seems to be a good indicator for urinary diseases in dogs.

KEY WORDS: canine, N-acetyl-β-D-glucosaminidase (NAG), urinary disease, urine.

N-Acetyl-β-D-glucosaminidase (NAG; EC 3.2.1.30) is a lysosomal enzyme found predominantly in the proximal renal tubular cells. Urinary NAG activity has been reported to increase under conditions of renal pathologic damage in both humans [2, 6, 8–10, 20] and other animals [1, 4, 15]. Urinary NAG activity also has been proposed as a sensitive marker of the progression of renal diseases [2, 6, 8, 9] and the rejection of renal allografts [13, 21]. Its activity increases prior to abnormal changes of other renal function test results [8]. The usefulness of urinary NAG activity values in dogs with drug-induced proximal tubular damage has been reported [4]. We reported the usefulness of measurements of urinary NAG activity in cattle [14, 15]. However, there is little information available about the measurement of urinary NAG activity in clinical cases of canine renal diseases.

In the present study, to clarify the usefulness of measurements of urinary NAG activity, we evaluated urinary levels of NAG in healthy dogs and dogs with urinary diseases.

MATERIALS AND METHODS

Animals: Twenty-eight healthy dogs (18 males and 10 females, 1–8 years old) and 20 dogs with urinary diseases (10 males and 10 females, 1–17 years old) were studied. The clinical conditions of the dogs with urinary disease are shown in Table 1, and included chronic renal failure (7 cases), lower urinary tract infection accompanied by pyelonephritis (2 cases), lower urinary tract infection without pyelonephritis (4 cases), diabetes mellitus (3 cases) and pyometra (4 cases). All of these dogs were sexually intact. Clinical diagnoses in diseased dogs were decided based on physical examinations, urinalysis, CBC, serum biochemistry, ultrasonography and radiography (plain and excretory urography).

Urine preparation: Untimed, freely caught urine samples were collected from the dogs. Urine samples were centrifuged at 500 × g for 5 min at 4°C, and NAG activity of the supernatants was measured. Analyses of NAG were performed within 7 hr of sample collection. In the dogs with chronic renal disease, urine samples were collected when BUN and serum creatinine concentration were less than 38.0 mg/dl and 2.0 mg/dl, respectively, and used for analysis of urinary NAG.

Measurement of urinary NAG activity and creatinine concentration: Urinary NAG activity was measured at 37°C, using a commercially available assay kit (Sanko Junyaku Co Ltd, Japan) with p-nitrophenyl N-acetyl-β-D-glucosaminide (PNP) as substrate and a spectrophotometer (Hitachi Ind., Japan) at 405 nm. The substrate concentration was 5 mM. Urinary concentration of creatinine was measured with the method described by Husdan and Rapoport [5]. Urinary NAG activity was expressed as units per liter, and the NAG index was calculated by the following equation:

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\text{NAG index (U/g) = urinary NAG activity (U/l)/urinary creatinine concentration (g/l)}
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Statistical analysis: Values of the urinary NAG index in healthy dogs are presented as mean ± SD. The urinary NAG index for healthy male dogs were compared with those for healthy female dogs by Student’s t-test. Correlations between BUN, serum creatinine concentration and urinary NAG index in dogs with chronic renal failure were also investigated.
RESULTS

Urinalysis revealed various changes in the dogs with chronic renal failure. Specific gravity and urine pH varied between 1.006–1.025 and 5.0–6.5. Protein was detected (2+ and 1+: 1+ to 4+ scale) in all urine samples. Squamous epithelial cells, transitional epithelial cells and renal tubular epithelial cells were detected (each one per several fields) in all urine samples, although granular casts were detected (one per several fields) in one dog. In dogs having lower urinary tract disease with and without pyelonephritis, blood (3+ and 2+: 1+ to 4+ scale) and protein (3+ and 1+: 1+ to 4+ scale) were detected in all urine samples. Bacteria were found in the urine of 3 dogs. Urine sediments from them contained WBC, RBC, squamous and transitional epithelial cells (several cells per each field). Urinary tubular epithelial cells were detected only in dogs having lower urinary tract disease with pyelonephritis. In diabetic dogs, urinary sugar and ketone bodies were detected in urine samples collected when blood sugar values were not well controlled. In dogs with pyometra, showing high values of NAG index, urinalysis revealed proteinuria (1+), and low specific gravity (1.003–1.005).

The mean values of urinary NAG index in healthy male and female dogs were 3.8 ± 2.6 U/g (0.3–9.8 U/g) and 2.1 ± 1.4 U/g (0.4–4.4 U/g), respectively. There were no significant differences between males and females. The overall mean value of urinary NAG index in healthy dogs was 3.2 ± 2.4 U/g (Table 1).

Urinary NAG index in dogs with chronic renal disease was 15.7 to 136.8 U/g, which was higher than that of healthy values. Values of BUN and serum creatinine concentration in these dogs were less than 38.0 mg/dl and 2.0 mg/dl, respectively. Thereafter, values of BUN and serum creatinine concentration showed an increase. Values of urinary NAG index in two cases were especially high (131.9 and 136.8 U/g). When the urine samples were collected, the values of BUN were 18.1 mg/dl and 34.7 mg/dl, respectively, and increased after that. In dogs with chronic renal failure, there was no significant correlation between urinary NAG index and BUN or serum creatinine concentration.

In two dogs with lower urinary tract infection accompanied by pyelonephritis, there were moderate increases of BUN (35.6–43.2 mg/dl) and serum creatinine concentration (0.6–1.1 mg/dl), however, the urinary NAG index was extremely elevated at 65.0 and 100.8 U/g. The value of NAG index in the case of a 2-year-old male dog was 61.6 U/g when its BUN and serum creatinine concentration were 35.6 mg/dl and 0.8 mg/dl, respectively. After that, NAG index, BUN and serum creatinine concentration increased to 100.8 U/g, 43.2 mg/dl and 1.1 mg/dl, respectively. In dogs having lower urinary tract infection without pyelonephritis, however, urinary NAG index showed normal values, except for two cases in which it was slightly increased. The NAG index did not increase during the monitoring period for cases of lower urinary tract disease without pyelonephritis, in contrast to the severe elevation of the NAG index in cases of lower urinary tract disease with pyelonephritis, as mentioned above.

In cases of diabetes mellitus, urinary NAG activity and index showed low values during the periods when control of blood sugar was well successful (Fig. 1). On the other hand, these values were elevated when blood sugar levels were high and urinary sugar and ketone bodies were detected.

Urinary NAG index varied from 0.4 to 48.0 U/g in cases of pyometra. Two dogs showed elevated values of urinary NAG index before ovariohysterectomy, and high levels of BUN and creatinine concentration were exhibited for 1–2 days after the operation (Fig. 2). BUN and creatinine concentration after ovariohysterectomy did not increase in the cases with low values of urinary NAG index, and these dogs showed a good course of recovery after the operation.

DISCUSSION

Little information is available about urinary NAG excretion in dogs and cats. In the present report, the values of uri-
Urinary NAG index in dogs with urinary diseases measured by using PNP as substrate are presented. Reusch et al. [12] reported that the mean urinary NAG index was 1.6 U/g (range: 0.03–11.2 U/g) in 10 healthy dogs, using 3 cresolsulphonephthaleinyl N-acetyl-β-D-glu-cosaminide as substrate. On the other hand, the mean urinary NAG index of healthy dogs measured using sodium-cresolsulphonephthaleinyl N-acetyl-β-D-glucosaminide as substrate was 5.7 ± 3.4 U/g in the report of Uechi et al. [18].

The mean urinary NAG index found in the present study was between those values with the caveat that there was a difference in the substrates used. Reusch et al. [12] also found higher levels of NAG index in male dogs than in female dogs. The reason for the higher activity of urinary NAG in males has been thought to be due to contamination of sperm cells, even when the urine samples are collected by cystocentesis [11]. In the present study, values of NAG index in males were also higher than those in females, however, the difference between urinary NAG index between the sexes was small, and there was no significant difference between them.

NAG excretion in urine is widely used as a marker of

Fig. 1. Changes of urinary NAG index, urinary sugar and ketone bodies in a canine case with diabetes mellitus. NAG index of urine samples, obtained when blood sugar was not well controlled and urinary sugar and ketone bodies were detected with high values.

Fig. 2. Urinary NAG index and BUN after ovariohysterectomy in canine cases with pyometra. Two of these cases manifested elevated values of NAG index before ovariohysterectomy, and also exhibited high levels of BUN for a few days after the operation.
tubular and glomerular injury in different pathological states of human diseases. Urinary NAG index has been reported as a sensitive indicator of early kidney injury and the rejection of renal allografts in human patients [13, 21], and has also been reported to be a valid marker for monitoring of progression in kidney diseases [2, 6, 8]. We previously reported that urinary NAG index was elevated in cows with renal parenchymal lesions [15]. In the present study, elevated values of urinary NAG index were also observed in dogs with renal diseases. Dogs with chronic renal failure showed an increase of NAG index before elevation of BUN and serum creatinine concentration. Furthermore, there were no significant correlations among BUN, serum creatinine concentration and urinary NAG index in these cases of chronic renal disease. It has been reported that there is no correlation between urinary NAG activity and serum creatinine concentration, and that urinary NAG excretion is more sensitive for early detection of renal damage than serum creatinine concentration in humans [8]. The results reported here for dogs with chronic renal failure are in agreement with those reported for humans.

In dogs with lower urinary tract infection, normal values of NAG index were observed except for cases of pyelonephritis. Two dogs with pyelonephritis showed extremely high values of NAG index. Elevated levels of urinary NAG activity were also reported in human cases of pyelonephritis [17]. The development of parenchymal lesions in the interstitium seems to cause damage to tubular cells resulting in NAG enzymuria. The increase of NAG index in the dogs with pyelonephritis also seemed to indicate tubular lesions, similar to the results found in affected humans. Therefore, monitoring the NAG index might be useful for predicting the complication of pyelonephritis in dogs with lower urinary tract infection. The existence of urinary sediments such as epithelial cells of the urinary tract and blood cells seemed to cause an increase in the urinary NAG index. In dogs with lower urinary tract infection, however, NAG index was not elevated. Urine samples of these dogs contained epithelial cells, WBC and RBC, and the moderate increase of cellular sediments as observed in this study seemed to have only a small influence on the values of NAG index in dogs. However, we could investigate only 4 cases of lower urinary tract infection without pyelonephritis in the present study. In canine lower urinary tract infection, further investigations of the relationship between urinary NAG index and urinary sediments is required to clarify this point. In addition, investigation of the effects of severe glucosuria on NAG measurement may be required.

It is known that there are many diseases in dogs which result in secondary renal damage, such as diabetes mellitus and pyometra. Dogs with diabetes mellitus showed high values of NAG index when blood sugar increased and urinary sugar and ketone bodies were detected. Urinary NAG index is used for the detection of early renal damage in human cases of diabetes mellitus [7, 22]. NAG enzymuria and microalbuminuria have been found to appear before macroalbuminuria is detected in human beings, and recent studies [7, 16, 19] recommended the measurement of urinary NAG as a marker for early diabetic nephropathy. In our canine cases of diabetes mellitus, high concentrations of urinary sugar seemed to cause damage to urinary tubular cells resulting in an increase of the NAG index. In the present study, we could not clarify whether urinary excretion of NAG increases before the dogs develop diabetic nephropathy, additional studies of dogs with diabetes mellitus from the early stages to the end stage will be required to clarify this. Monitoring of NAG index, at least, seemed to be useful for detection of tubular damage induced by severe glucosuria.

Secondary renal disease is also known to be induced by endotoxemia in canine pyometra. Dogs that had pyometra and exhibited renal insufficiency after ovariohysterectomy showed high values of NAG index before ovariohysterectomy. Furthermore, dogs with low values of NAG index before and after ovariohysterectomy maintained normal renal functions after ovariohysterectomy. Therefore, urinary NAG index may be a good indicator of secondary renal disease in canine pyometra. In the present study, however, we studied only two cases with high levels of NAG index. Additional studies in dogs with pyometra will be required to determine the usefulness of NAG index as an early indicator of renal disease in pyometra.

From the results reported here, measurement of urinary NAG seemed to yield information about tubular damage at an early stage in dogs with urinary diseases, as was shown earlier in human cases.

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REFERENCES


