

Evaluation of Urinary Enzymes in Dogs with Early Renal Disorder

Masami UECHI, Yusuke NOGAMI, Haruko TERUI, Tomohiro NAKAYAMA, Ryoukichi ISHIKAWA, Yoshito WAKAO, and Mitsugi TAKAHASHI

Department of Veterinary Surgery, School of Veterinary Medicine, Azabu University, Sagami-hara-city, Kanagawa 229, Japan

(Received 22 April 1993/Accepted 16 December 1993)

ABSTRACT. We investigated urinary N-acetyl- β -D-glucosaminidase NAG (EC 3.2.1.30), γ -glutamyl transpeptidase γ -GTP (EC 2.3.2.2) and glycyl-prolyl dipeptidyl aminopeptidase GP-DAP (EC 3.4.14.5) in dogs with heartworm disease and renal failure. In the renal failure dogs, the NAG, γ -GTP and GP-DAP index were significantly higher than those in the healthy dogs. In the heartworm disease dogs with normal chest X-rays (HW I), none of the enzyme values was significantly different from those of the healthy controls. In the dogs with heartworm disease showing abnormal heart shadows on their chest X-rays (HW II), enzyme values were significantly higher than those in the healthy dogs ($P<0.01$) and the HW I dogs ($P<0.01$). Thus, these urinary enzymes tests are available for the early detection of renal disorders.—**KEY WORDS:** heartworm disease, renal failure, urinary enzyme.

J. Vet. Med. Sci. 56(3): 555–556, 1994

The kidney has high compensatory capacity [1]. Although the kidney of dogs with early renal disorders shows histopathological changes such as glomerulonephritis, interstitial nephritis and tubular nephropathy, general renal function tests (serum creatinine, serum urea nitrogen, and endogenous creatinine clearance) show normal values [4, 9, 10]. However, increased urinary enzyme activity has been detected in gentamicin induced nephropathy, even though the results of general renal function tests were normal [6], suggesting that urinary enzymes would be useful in the detection of early renal disorders.

The present study was aimed at the detection of early renal disorders developed in heartworm disease by analysis of urinary enzymes. Urinary N-acetyl- β -D-glucosaminidase (NAG, EC 3.2.1.30), γ -glutamyl transpeptidase γ -GTP (EC 2.3.2.2) and glycyl-prolyl dipeptidyl aminopeptidase (GP-DAP, EC 3.4.14.5) activities were measured in the dogs with renal failure and heartworm disease.

In this experiment, 9 renal failure dogs (7 males and 2 females, B.W. 6.1–17.6 kg) with clinical signs and elevated serum creatinine (S-Cr, 1.5 mg/dl<) and urea nitrogen (SUN, 50 mg/dl<) respectively (Table 1). And 18 dogs (B.W. 6–22 kg) with naturally acquired heartworm disease (HW) diagnosed by the detection of heartworms in their pulmonary arteries were used. The HW dogs were divided into two groups based on their chest X-ray finding: an HW I group (n=9) with normal cardiac shadows and an

HW II group (n=9) with enlarged main pulmonary arteries, embolized pulmonary arteries and an enlarged right ventricle. There were no clinical signs in the HW I group, but the HW II group exhibited weakness and a cough. The S-Cr and SUN levels in both groups were within normal limits (Table 2). The clinically healthy 17 dogs (7 males and 10 females, B.W. 7–13 kg) with normal routine biochemical and urinalysis values were also examined as control.

Urine samples were collected by sterile urethral catheterization as random urine samples from 9:00 to 16:00. The urine samples were centrifuged at 3,000 g for 5 min, the supernatant was stored at 4°C, and urinary enzymes were measured within 48 hr.

The NAG activity was measured with a commercial test kit using sodio-m-cresolsulfonphthaleinyl-N-acetyl- β -D-glucosaminide (MCP-NAG) as the substrate at 37°C and 580 nm [11], and the GP-DAP activity was measured kinetically with Gly-Pro-3.5-dibromo-4-hydroxyanilide as

Table 1. Laboratory findings of dogs with renal failure

	Sex	B.W.	Age	Cr	BUN	GOT	GPT	ALP
No. 1	♂	12.0	13.0	2.2	85.0	39	19	415
No. 2	♂	6.1	7.0	12.0	276.0	—	—	—
No. 3	♂	9.5	12.0	3.0	140.1	39	54	95
No. 4	♂	10.2	6.0	10.7	243.0	35	52	—
No. 5	♂	17.6	11.0	2.7	76.3	257	247	2107
No. 6	♂	16.7	3.0	2.3	64.2	55	94	141
No. 7	♂	10.0	—	3.0	75.0	—	—	—
No. 8	♀	17.0	9.0	2.5	89.4	35	25	333
No. 9	♀	10.7	10.0	4.7	161.1	151	138	724

B.W. is body weight (kg), Cr (mg/dl), BUN (mg/dl) GOT (U/l), GPT (U/l), ALP (U/l).

Table 2. Laboratory findings of dogs with heartworm disease with normal (HW I: No. 1–9) and abnormal (HW II: No. 10–18) cardiac shadow by chest X-ray

	Sex	B.W.	Cr	BUN	GOT	GPT	ALP
No. 1	♂	7.6	0.8	17.5	25	34	132
No. 2	♀	7.1	0.9	11.8	38	45	142
No. 3	♀	9.0	0.5	19.1	47	58	—
No. 4	♀	10.0	0.2	11.9	35	52	—
No. 5	♂	17.0	1.4	21.0	14	21	135
No. 6	♂	11.0	0.9	15.1	23	35	—
No. 7	♀	12.0	0.9	15.3	53	35	—
No. 8	♂	10.6	0.6	8.2	75	128	140
No. 9	♂	10.0	0.9	25.1	35	42	—
No. 10	♂	22.0	1.4	9.8	109	—	305
No. 11	♀	6.5	0.5	14.3	54	39	195
No. 12	♀	14.0	1.0	12.0	99	205	—
No. 13	♂	14.0	0.8	6.1	70	38	195
No. 14	♂	11.0	0.9	19.8	47	129	240
No. 15	♀	10.0	0.8	15.1	70	129	239
No. 16	♀	10.0	1.1	22.4	44	41	74
No. 17	♀	13.0	0.8	9.5	21	54	—
No. 18	♀	6.0	0.8	37.6	114	183	—

Cr (mg/dl), BUN (mg/dl), GOT (U/l), GPT (U/l), ALP (U/l).

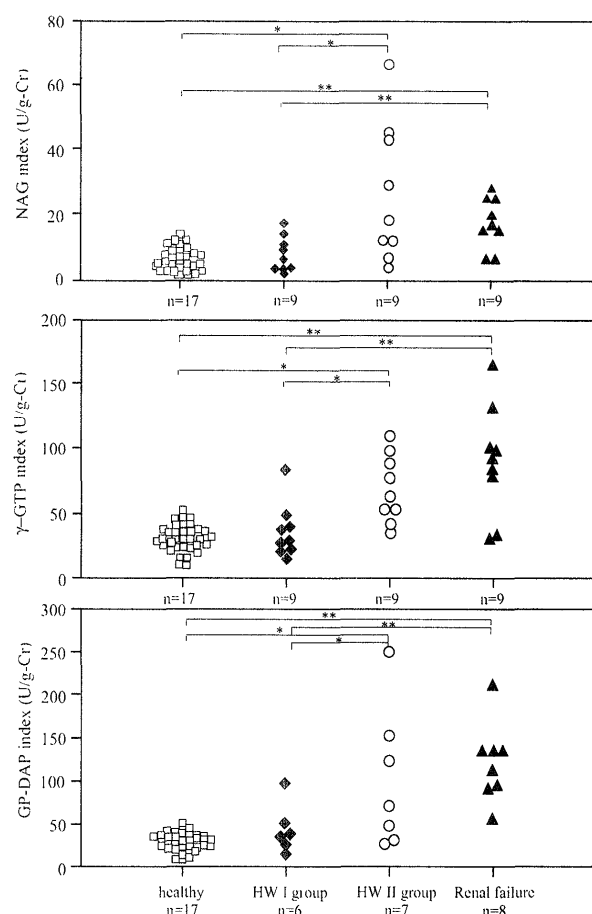


Fig. 1. Comparison of urinary enzymes in the dogs with heartworm disease and renal failure. *: compared with healthy dogs ($P<0.01$) and HW I group ($P<0.05$), **: compared with healthy dogs and HW I group ($P<0.01$).

the substrate at 37°C and 600 nm [14]. Both enzymes were analyzed using a DU-8 Spectrophotometer (Beckman, U.S.A.). Urine γ -GTP activity was measured with commercial test kit utilizing γ -glutamyl-p-nitroanilide as the substrate at 37°C and 405 nm using RaBA Σ (Chugai Pharmaceutical Co., Ltd., Japan). Serum and urine creatinine were measured by the Jaffe reaction method. Urinary enzyme activities were corrected based on urine creatinine concentrations and expressed as the creatinine index (U/g-Cr).

Statistical analysis was performed by means of the Kruskal-Wallis and Mann-Whitney tests. Significant differences were considered to exist at $P<0.05$.

Figure 1 shows NAG, γ -GTP and GP-DAP index for each animals. No significant difference in these 3 enzyme indices were observed between the healthy dogs (NAG: 5.7 ± 3.4 U/g-Cr, γ -GTP: 30.4 ± 10.0 U/g-Cr, and GP-DAP: 29.3 ± 10.5 U/g-Cr) and the HW I group (NAG: 6.9 ± 5.5 U/g-Cr, γ -GTP: 34.5 ± 20.4 U/g-Cr, and GP-DAP: 41.7 ± 28.7 U/g-Cr). However, the HW II group (NAG: 25.8 ± 21.1 U/g-Cr, γ -GTP: 90.4 ± 81.3 U/g-Cr, and GP-DAP: 99.9 ± 80.5 U/g-Cr) and renal failure dogs (NAG: 17.1 ± 7.9 U/g-Cr, γ -GTP: 89.6 ± 42.9 U/g-Cr, and

GP-DAP: 121.0 ± 45.4 U/g-Cr) had significantly higher values than the healthy dogs ($P<0.01$ and $P<0.01$, respectively) and the HW I group ($P<0.05$ and $P<0.01$, respectively).

The kidney needs high oxygen supply and renal blood flow for high metabolism *i.e.* reabsorption of glucose, amino acid and protein from the glomerular fluid to the blood [2, 7]. Reduced renal blood flow cause acute renal failure [7]. Poor cardiopulmonary function in dogs with serious heartworm disease may lead to tubular ischemia and necrosis [8, 10, 12]. In the present study, although urinary enzymes were normal range in the HW I group with normal cardiac shadow by chest X-ray, these values were significantly increased in the HW II group with abnormal cardiac shadow. The increased urinary enzymes mainly originated in proximal tubule cells and indicated renal tubular damage [3, 5, 13]. These results suggest that urinary enzyme levels in random urine samples are available for detection and monitoring of early phase renal disorders.

ACKNOWLEDGEMENT. The authors wish to thank Mr. Yoshitada Saito and Mr. Hiroshi Miyasaka of Fujirebio Inc. for GP-DAP analysis kit.

REFERENCES

1. Boveé, K. C. and Joyce, T. 1979. *J. Am. Vet. Med. Assoc.* 174: 488–491.
2. Berry, C. A. and Rector, F. C. Jr. 1991. pp. 245–282. *In: The Kidney*, 4th ed. (Brebber, B. M. and Rector, F. C. Jr. eds.), W. B. Sanders, Philadelphia.
3. De Schepper, J., De Cock, I., and Capiiau, E. 1989. *Res. Vet. Sci.* 46: 396–400.
4. Finco, D. R. and Duncan, J. R. 1976. *J. Am. Vet. Med. Assoc.* 168: 593–601.
5. Gosset, K. A., Turnwald, G. H., Kearney, M. T., Greco, D. S., and Cleghorn, B. O. 1987. *Am. J. Vet. Res.* 46: 2332–2335.
6. Gouyon, J. B., Aujard, Y., Abisror, A., Laudignon, N., d'Athis, P., Jacqz, E., Biou, D., Demelier, J. F., and Mathieu, H. 1987. *Dev. Pharmacol. Ther.* 10: 145–152.
7. Hays, S. R. 1992. *Am. J. Med. Sci.* 304: 93–108.
8. Kitagawa, H., Kubota, A., Yasuda, K., Hirano, Y., and Sasaki, Y. 1992. *Jpn. J. Vet. Sci.* 54: 751–756.
9. Knghit, D. H. 1987. pp. 1463–1518. *In: The Veterinary Clinics of North America: Small Animal Practice*. 17 (Kreive, R. B. ed.), W. B. Sanders, Philadelphia.
10. Ludders, J. W., Grauer, G. F., Dubielzig, R. R., Ribble, G. A., and Wilson, J. W. 1988. *Am. J. Vet. Res.* 49: 826–830.
11. Noto, A., Ogawa, Y., Mori, S., Yoshioka, M., Kitakaze, T., Hori, T., Nakamura, M., and Miyake, T. 1983. *Clin. Chem.* 29: 1713–1716.
12. Osborne, C. A., Hammer, R. F., O'Leary, T. P., Pomeroy, K. A., Jeraj, K., Barlough, J. E., and Vernier, R. L. 1981. pp. 67–92. *In: Proceedings of the Heartworm Symposium '80* (Otto, G. F. ed.), Veterinary Medicine Publishing Co., Kansas.
13. Price, R. G. 1982. *Toxicology* 23: 99–134.
14. Takasawa, M., Kasahara, S., Ikarashi, K., Nakamura, H., Tuda, A., Ito, S., and Shibata, A. 1990. *J. Jpn. Diab. Soc.* 33: 287–291 (in Japanese).