นิพนธ์ต้นฉบับ

Urinary enzymes in healthy adults, and in dogs treated with Russel's viper venom.*

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Buranasiri K, Tosukhowong P, Thamaree S, Chaiyabutr N, Sitprija V. Urinary enzymes in healthy adults, and in dogs treated with Russel's viper venom. Chula Med J 1987 Jan ; 32 (1) : 31-36

Urinary activities of enzymes N-acetyl-β-glucosaminidase (NAG), β - galactosidase (GAL), γ - glutamyl transpeptidase (GGT) and alanine aminopeptidase (AAP) were determined in 106 healthy adults, 51 males and 55 females. Colorimetric methods were used and the results were measured in international units per gram creatinine. In the male the mean enzyme activities were NAG = 3.28±1.74, GAL = 1.88±1.60, GGT = 15.29± 6.94, AAP = 12.52±5.43 and in the female, NAG = 4.01±1.41 GAL = 2.13±1.13, GGT = 20.54±5.73 and AAP = 11.13±3.50. The mean activities of NAG and GGT in female were statistically higher than those in the male ($p < 0.01$), but there were no differences in their AAP and GAL activities. There was an elevation of NAG level in dogs after the infusion of Russel's Viper venom, ($p < 0.005$).

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Received for publications. January 1, 1987

* Supported by a grant from the Rachadapiseksompoj-China Mediacal Board Research Fund.
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การศึกษาระดับในปัสสาวะของเอ็นไซม์ N-acetyl-β -D-glucosaminidase (NAG), β -galactosidase (GAL), γ - glutamyl transpeptidase (GGT) & alanine aminopeptidase (AAP) ในคนที่มีสุขภาพดี จำนวน 106 คน เป็นชาย 51 คน หญิง 55 คน โดยใช้วิธี colorimetric ได้ค่าเฉลี่ยของเอ็นไซม์คิดเป็นยูนิตสากลต่อกรัมของครีเอตินินดังนี้

ในชาย NAG = 3.28±1.74, GAL = 1.88±1.60, GGT = 15.29±6.94

AAP = 12.5±5.43

ในหญิง NAG = 4.01±1.41, GAL = 2.13±1.13, GGT = 20.54±5.73 &

ค่าเฉลี่ย NAG และ GGT ในหญิงสูงกว่าในชาย ($p<0.01$) ส่วนค่า GAL และ AAP ของชายและหญิงไม่แตกต่างกันตามนัยทางสถิติ ในสุนัขที่ได้รับการฉีดพิษงูแมวเซาพบว่าจะมีค่า NAG สูงขึ้นอย่างมีนัยสำคัญ ($p < 0.005$)

One of the characteristics of the kidney is the ability to compensate for damage. Classical function tests are therefore insensitive for detecting small, unknown early renal damage. Renal biopsy is of diagnostic value but requires skilled performer and pathologist. Above all renal biopsy is too invasive and may cause harm to the patient. The presence of enzymes in urine has long been known and their use as diagnostic indicators of renal disease has been proposed for a considerable time.[1] During the last two decades, significant technological development has made enzyme assays much more simple, rapid and reproducible. Thus urinary enzyme assay has received more attention as a diagnostic approach recently, as can be seen from reviews by Raab,[1] Price[2] and Vanderlinde.[3]

Although more than 40 urinary enzymes have been studied, only a few have gained interest as diagnostic indicators. These include N-acetyl-β-D-glucosaminidase (NAG), E C.3.2.1.30 and alanine aminopeptidase (AAP), E.C.3.4.11.2. The diagnostic potential of urinary enzymes is often enhanced by the simultaneous assay of more than one enzymes, particularly if the the activities of the enzymes used are high in different regions of the nephron. We therefore have chosen to study urinary excretion of two lysosomal enzymes, NAG and GAL (β-galactosidase, E.C.3.2.1.31) and two brush border enzymes, AAP and GGT (γ-glutamyl transpeptidase, E C.2.3.2.2.) in healthy adults.

Material and Methods.

Three hour morning urine was collected from healthy adult subjects with no history of drug taking a week before the experiment. All urine samples must give negative result to Combur-9 (Boehringer), an urinary strip test for protein, glucose, bilirubin, urobilinogen, ketone bodies, leucocyte, erythrocyte and hemoglobin. All Subjects were medical or nursing students or medical personels.

Ten ml. of urine was centrifuged at 3000× g for 5 minutes. Centrifuged urine, 3 ml, were subjected to gel filtration on Sephadex G-50, fine (Pharmacia) as described in detail by Werner.[4] The first 3 ml of the column effuent were discarded. The following 6 ml. were collected in bulk and analyzed for enzyme activities at once or not later than 2 days, kept at 4.C.

NAG and GAL were assayed as described by Maruhn,[5]. The AAP assay method was after Jung and Scholz[4], the kinetic assay having been modified to a colorimetric one. The reaction was allowed to occur for 15 minutes a 37.C then stopped with AMP buffer (pH 10.25), which was also used in NAG and GAL determinations. The absorbance was measured at the same wavelength as other enzyme assays, 410 nm. The method of Jacobs[6] was used for GGT determination. Urine creatinine was determined according to Bonsnes and Taussky.[7]

Chemicals used unless stated were from Sigma Chemicals.

Results

Coefficient of variations (C.V.) calculated from simultaneous repeated assays of the same samples for the four enzymes were shown in table 1. Shown also in this table were C.V. of repeated determinations of the same sample kept at 4° C every day for 16 consecutive days. No tendency for deterioration was observed during this stored period.

Urine samples were collected from 51 men, 17-56 years of age and 55 women, 19-50 years. Enzyme activities were tabulated in table 2. Mean NAG and GGT activities were statistically higher in women than in men (student t-test), if expressd per urine creatinine-weight.

Table 1 Precision data

within run	n	$\bar{X}$ U/L	S.D.	C.V. %
NAG	12	2.50	0.04	1.61
GAL	10	1.63	0.06	3.68
GGT	10	62.32	1.86	2.98
AAP	10	13.51	0.97	7.19
between run				
NAG	16	3.17	0.13	4.10
GAL	16	1.42	0.17	11.97
GGT	16	5.11	0.56	10.96
AAP	16	2.81	0.26	9.11

n = number of assay

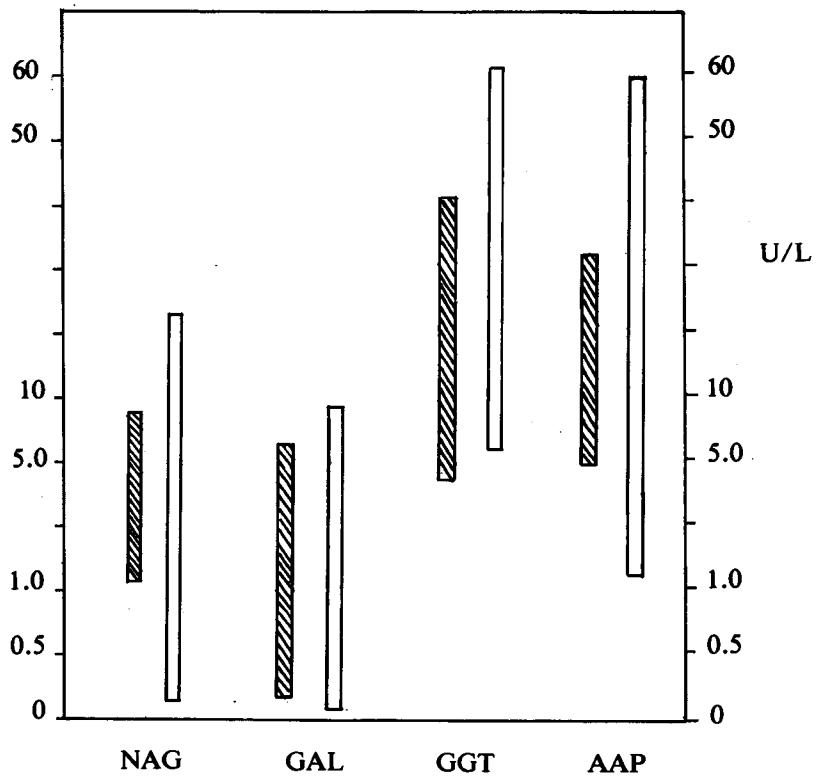

Figure 1 Normal Range of Enzyme Activities (male).

Activity in U/g creatinine Activity in U/L

Table 2 Enzyme activities.

	U/L			U/G creatinine			
	mean	**S.D.**	**range**	**mean**	**S.D.**	**range**	**p value***
			I NAG activity				
male	5.87	4.67	0.22-21.62	3.28	1.74	0.6-8.69	
female	5.86	3.94	0.86-16.65	4.01	1.41	1.86-6.9	<0.01
			II GAL activity				
male	2.99	3.06	0.22-10.27	1.88	1.60	0.1-6.98	
female	3.04	2.30	0.11-10.27	2.13	1.13	0.08-5.54	N.S.
			III GGT activity				
male	25.23	17.93	5.48-63.02	15.29	6.94	4.83-43.84	< 0.01
female	28.43	15.88	8.22-65.76	20.54	5.73	5.74-30.9	
			IV AAP activity				
male	21.25	14.96	2.05-64.68	12.52	5.43	5.15-32-34	N.S.
female	16.22	11.21	2.05-51.33	11.13	3.50	4.63-19.29	

We also reported the effect of Russel's Viper venom (RVV) on urinary excretion of these enzymes. Aneshetized healthy dogs were used RVV was infused intravenously 50 μg/kg. at the rate of 0.5 ml/min. Urine samples were collected before and after infusion as shown in protocal table 3. In table 3. there were some rises in activities of all enzymes studied at some time after the venom infusion. The increasing activities of NAG with time were stepwise and statistically significant ($p < 0.005$). Although other enzymes showed some activity changes but owing to a small number of dogs studied, no conclusion could be reached from the data.

Table 3 Enzyme activities before and after snake venom administration.

Treatment	Control	Experiment		After
	NSS	RVV 50 Ug/kg		NSS
time of urine collection (min)	0-20	20-40	40-60	60-80
NAG activity μU/L	727±301 (n = 7)	1093±687 (n = 5)	1323±525 (n = 6)	1484±386 (n = 7) * $p < 0.005$
GGT activity μU/L	1989±653 (n = 6)	1848±660 (n = 6)	1763±586 (n = 6)	2299±441 (n = 5)
AAP activity μU/L	887±473 n = 6	1661±437 n = 4	746±325 n = 6	753±233 n = 6
GAL activity μU/L	103±65 n = 3	124±57 n = 2	134±95 n = 3	411±357 n = 3

NSS = normal salive solution
RVV = Russel's viper venom
n = number of subjects
* = student t-Test

Discussion

In this report the activities of four urinary enzymes were assayed by colorimetric method which was simple, rapid and reproducible (table 1), The enzymes appeared to have wide ranges of activities and diurnal variations.[2,8] However, the range of activities was shown to be relatively narrow when corrected with urinary creatinine (Figure 1).

Enzyme activities of the Thai adults in this report can hardly be compared with those of other nationalities because of the difference in assay methods and in unit expression. In Germany, Maruhn[5] using the same method reported NAG acivities of 1.5-29.8 U/L and GAL of 0.1-16.8 U/L which were higher than those in this report. The GGT activity in Hungarian children was reported to be lower[9]. For AAP, its activity was reported by Jung and Scholz[8], using a small number of subjects, to be lower, Urinary levels of 11 enzymes were reported using transforming factors for the final calculation[10] and therefore could not be compared to those in this report.

NAG level appeared to agree well with an other report in Thailand[11] which showed no sex difference. However, NAG and GGT activities in females were higher than those in males in this report, and comparable to those reported by Maruhn[10]. There was no sex diference in AAP and GAL activities, which agreed with the results by Jung[8] and Maruhn[5]. Other urinary enzymes beside NAG have not been studied in Thai subjects before.

NAG and GAL are lysosomal enzymes with high activities in the proximal renal tubules. In plasma their activities are low. Although AAP and GGT are rich at the same site they are brush border membrane enzymes. The plasma AAP is not the same enzyme as the urinary AAP. Moreover enzymes in plasma can not pass through the glomerular membrane because of their large molecular size. NAG and GGT have molecular weights of 130,000-140,000 and 80,000-90,000 respectively.[3,12] From the NAG isozyme study, it is evident that serum and urine enzymes are not the same[13]. Therefore a rise in urinary levels of these enzymes reflects the presence of injury to renal tubular cells. Thus the determination of urinary enzymes has a great benefit

in diagnosis, treatment and prognosis of renal disease as well as in the early detection of renal transplant rejection, and of drug and heavy metal nephrotoxicity.[2,14,15,16,17,18,19,20] There have been some reports of increased enzyme levels in non renal diseases e.g. primary hypertension, hyperthyroidism and infective hepatitis[2] In interpreting changes in enzyme activities these conditions have to be taken into consideration.

The stepwise increase in NAG activities (Table 4) revealed some renal parenchymal effects of RVV in dogs. Other enzyme activities did not show significant changes. Owing to a small number of animals studied, further work should be performed using more subjects.

References

1. Raab WP. Diagnostic value of urinary enzyme determination. Clin Chem 1972 Jan; 18(1) : 5-25
2. Price RG. Urinary enzymes, nephrotoxicity and renal disease. Toxicology 1982, 23(2-3) : 99-134
3. Van der Linde RE. Urinary enzyme measurements in the diagnosis of renal disease. Ann Clin Lab Sci 1981; 11(3) : 189-201
4. Werner M, Maruhn D, Atoba M. Use of gel filtration in the assay of urinary enzymes. J Chromatogr 1969 Mar; 40(3) : 244-263
5. Maruhn D. Rapid colorimetric assay of β-galactosidase and N-acetyl-β-glucosaminidase in human urine. Clin Chim Acta 1976; 73(3) : 453-461
6. Jacobs WLW. A colorimetric assay for γ-glutamyl transpeptidase. Clin chim Acta 1971; 31(1) : 175-179
7. Varley H. Practical Clinical Biochemistry. 4th ed. London : William Heinemann Medical Books, 1967. 197-198
8. Jung K, Scholz D. An optimized assay of alanine aminopeptidase activity in urine. Clin Chem 1980 Aug; 26(9) : 1251-1254
9. Salgo L, Szabo A. Gamma-glutamyl transpeptidase activity in human urine. Clin Chin Acta 1982 Nov; 126(1) : 9-16
10. Maruhn D, Fuchs E, Mues G, Bock KD. Normal limits of urinary excretion of eleven enzymes. Clin chem 1976 Oct; 22(10) : 1567-1574
11. Ong-aj-yooth S, Ong-aj-yooth L, Nilwarangkur S. The serum levels and urinary excretion of NAG in normal Thai adults. Ann Intern Med 1986, 2(1) : 5-8
12. Rambabu K, Jacob RT, Pattabiraman TN. Variants of gamma-glutamyl transferase in human urine. Clin Chim Acta 1981 Feb; 109(3) : 257-265
13. Gibey R, Dupond JL, Henry JC. Urinary N-acetyl-bata-D-glucosaminidase (NAG) isozyme profiles : a tool evaluating nephrotoxicity of aminoglycosides and cephalosporins. Clin Chim Acta 1984 Feb 14; 137(1) : 1-11
14. Whiting PH, Nicholls AJ, Catto GRD, Edwards H, Engeset J. Patterns of N-acetyl-beta-D-glucosaminidase excretion after renal transplantation. Clin Chim Acta 1980 Nov 20; 108(1) : 1-7
15. Wellwood JM, Ellis BG, Hall JH, Robinson D, Thompson AE. Early warning of rejection?. Br Med J 1973 May 5; 2(5861) : 261-265
16. Thompson AE, Tucker SM, Price RG. Assay of N-acetyl-beta-D-glicosaminidase in urine of patients with renal transplants as an indicator of rejection. Clin Chem 1977 Feb; 23(2 ptl) : 301-302
17. Wellwood JM. Ellis RG, Price RG, Hammond AE. Urinary N-acetylbeta-D-glucosaminidase activities in patients with renal disease. Br Med J 1975 Aug 6; 3(5978) : 408-411
18. Adelmann RD, Spangler WL, Beasom F, Ishizaki G, Conzelman GM. Furosemide enhancement of experimental gentamicin nephrotoxicity : comparison of functional and morphological change with activities of urinary enzyme. J Infect Dis 1979 Sep; 140(3) : 342-352
19. Gibey R, Dupond JL, Alber D, Leconte des Floris R, Henry JC. Predictive value of urinary N-acetyl-beta-D-glucosaminidase (NAG), alanine-amino-peptidase (AAP) and beta-2-microglobulin (β_2-m) in evaluating nephrotoxicity of gentamicin. Clin Chim Acta 1981 Oct 8; 116(1) : 25-34
20. Burchardt U, Haschen RJ, Krosch H. Clinical usefulness of enzyme determinations in urine. Curr Probl Clin Biochem 1979; (9) : 106-112