Research Article

N-ACETYL-β-D-GLUCOSAMINIDASE IN URINE OF PATIENTS WITH CIRRHOSIS

Duan Yong[1]
Zhou Zhenfen[2]
Wang Yuming[1]
Zhang Qiuxia[1]
Li Shuan[2]

Abstract

N-acetyl-β-D-glucosaminidase (NAG) was studied in urine from 44 patients with cirrhosis and 86 healthy control subjects. NAG activity was significantly higher in the cirrhosis group than in the normal control group (46.0 ± 30.8 *vs.* 6.3 ± 2.5 U/g. creatinine; $p < 0.01$); NAG activity presented a progressively increase along with severity of liver disease graded as A→B→C ($p < 0.01$) by the classification of liver function. The urinary NAG may provide additional diagnostic information monitoring early renal damage of cirrhotic patients and possesses great significance in the indirect judgment and prognosis of liver function lesion and its severity.(Bull Chiang Mai AMS 1996; 29:69-71)

Key words : Liver cirrhosis, renopathy, NAG

Departments of [1]Medical Laboratory and [2]Gastroenterology, First Affiliated Hospital, Kunming Medical College, Kunming 650032, People's Republic of China

Glomerular impairment is quite common in patients with hepatic cirrhosis, but the damage usually takes on a latent status, and seldom clinical symptoms in the kidney. It is too difficult to diagnose. When the renal function test *i.e.* BUN and creatinine, are abnormal the patients condition is usually unreversible. Therefore, it is urgent that we should find out a diagnostic method for detection of early cirrhotic renal impairment. N-acetyl-β-D-glucosaminidase (NAG; EC 3.2.1.30), together with other lysosomal hydrolases, acts to degrade glycoproteins and mucopolysaccharides, substances implicated in the development of microangiopathy in some renal damages. Up to now there were few research reports on this problems. We measured urinary NAG in 44 patients with hepatic cirrhosis from June 1993 to April 1994. Consequently, we have attempted to establish urinary NAG which may provide additional diagnostic information for monitoring early renal damage of cirrhotic patients.

Materials and Methods

Subjects : Urine specimens were collected from 44 cirrhotic patients, 31 males and 13 females, aged 20-58 (average 48.9) years old. Among these, 33 cases were posthepatic cirrhosis, 7 cases were alcoholic cirrhosis, 3 cases were schistosomal cirrhosis and 1 case was miscellaneous. Urine from 86 healthy control subjects, 64 males and 22 females, aged 15-56 (average 44.5) years

old were also collected. All patients and controls had normal renal function tests with negative urine routine examination, normal levels of BUN and creatinine, no renal disease history by other pathogenic causes. On the first day, the patients and controls were given neither oily food nor medicine and urine specimens were collected on the second day.

The cirrhotic patients were divided according to severity of liver disease into 3 groups, A, B and C by Trey, *et al* as cited by Pugh, et al (1).

Reagents : All chemicals used were of analytical grade: 2-chloro-4-nitrophenol-N-acetyl-β-D-glucosaminide(CNP-NAG) and 2-chloro-4-nitrophenol (CNP) were from Tianjing Institute of Pharmaceuticals, China.

Enzyme assay : NAG activity in urine was measured by the method of Makise J, *et al*[2] by incubating 20 μL of urine with 240 μL of citrate buffer (100 mmol/L, pH 4.8) containing 2.0 mmol of CNP-NAG per liter as substrate at 37 °C for 6 minutes on Monarch 1000 Automatic Biochemical Analyzer (US, IL). NAG activity was calculated by CNP formation rate with the change in absorbance per minute at 405 nm. Under these condition, the amount of CNP liberated was linearly related to time for 400 seconds, up to a maximum activity of 411 U/L. The between-run CV were 3.46% and 3.60% for mean activities of 109.73 U/L (n = 20) and 5.88 U/L (n = 10), respectively. The within-run CV (n = 10) were 3.58% and 6.97% for mean activities of 35.48 U/L and 2.44 U/L. respectively. One unit (U) of enzyme activity is defined as that which liberates 1 μmol of CNP per minute. We expressed NAG activity in terms of urinary creatinine, *i.e.* U/gram of urinary creatinine. Urinary creatinine was measured by the Jaffe method as modified by our laboratory.

For statistical analysis, t-test and rank sum test were used for comparison among multiple groups (H test).

Results

Urinary NAG activity in cirrhosis patients was significantly higher than in normal subjects. (46.0 ± 30.8 *vs.* 6.3 ± 2.5 U/g creatinine, $p < 0.01$) as shown in Table 1.

Comparison of urinary NAG activity with classification of severity of liver dysfunction for cirrhosis was performed by H test. NAG activity presented a progressively increased from A→B→C ($p < 0.01$) as shown in Table 2

Table 1 Urinary NAG (U/g creatinine) of Cirrhotic Patients and Control Subjects

Group	n	NAG
Control	86	6.3 ± 2.5
Cirrhosis	44	46.0 ± 30.8
p		< 0.01

Table 2 A Comparison of Urinary NAG by Different Liver Function Levels.

Levels	n	NAG
Child A	12	17.7 ± 3.9
Child B	22	49.0 ± 26.7
Child C	10	85.2 ± 10.6
H		16.7
p		< 0.01*

* When any two variables in NAG group compared, $p < 0.05$

Discussion

Kidney impairment is a common complication of liver cirrhosis. The early impairment is being recessive in stage and shows no clinical symptoms, which is difficult to diagnose. With the development of disease course the injury get worse, so the early diagnosis is necessary. Literature available had presented some accounted that the latent injury lies in the early stage of 75% of patients with cirrhotic renopathy. In some circumstances, nevertheless, such factors as drug nephrotoxicity, infections, shock and dehydration, are likely to induce renal dysfunction, to which a full attention should be paid in the clinical work so that early prevention and treatment can be performed[3]. The mechanism of cirrhotic renal impairment is incompletely known. Our possible inference is that with the recession of liver function, there appeared a collateral circulation, which in turn change the hemodynamic circulation of blood and resulted in the precipitation of such vasoactor materials as polymeric IgA and circular immunization IgA compounds.

NAG is a hydrolytic enzyme (Mr = 130,000-140,000) which is widely distributed in various tissues and is poorly filtered in the normal glomeruli. In the kidney it is primarily located in the lysosomal fraction and to a much lesser extent in the microsomal fraction of the tubular epithelial cells. Lysosomal enzymes may enter the urine without cell destruction. When proximal renal tubules are stressed and injured, excretion of these enzymes will increase in the urine. So, the NAG activity is a useful marker for early diagnosis of renal damage(2).

In our cirrhotic patients the most significant observation is the elevated NAG activity, which is positively related to the degree of the liver injury and is correspondence with medical literature published(4,5). Consequently, the assay of NAG activity is not only a sensitive marker for monitoring early renal impairment of cirrhotic patients, but highly valuable in the indirect diagnosis and prognosis of the severity of cirrhotic liver injury. Moreover, since NAG determination is easy to perform, it can also be recommended as a routine follow-up test for cirrhotic patients.

References

1. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 646-9.
2. Makise J, Salto E, Obuchl M, et al. Kinetic rate assay of urinary N-acetyl-beta-D-glucosaminidase with 2-chloro -4-nitrophenol-N-acetyl-beta-D-glucosaminide as substrate. Clin Chem 1988; 34: 2140.
3. Qi D, Fu X, Chen J, Zhao H. Renal function change in 40 patients who had liver fibrosis but had no ascites fluid. Chinese Internal J 1994; 33:623.
4. Amakasu H, Kanno A, Abe M, Sato K, Goto Y. Urinary N-acetyl-beta-D-glucosaminidase activity in liver cirrhosis with renal impairment. Nippon Shakakibyo Gokkai Zasshi (Japan) 1991; 88: 55.
5. Bruno CM, Raciti C, Urso G, Neri S, Noto R. Enimi urinaty in corso di cirrosi epatica : utili markers precoci di anno renale. Minerva Med 1994; 85: 155.

Research Article

FACTORS AFFECTING ON WASTEWATER ANALYSIS

Suchada Chaisawadi,*
Usa Jeenjenkit,*
Sopida Khotaraveera*

ABSTRACT

This paper shows how to analyse the laboratory parameters of wastewater effectively. Wastewater samples were taken from a tapioca factory and then analysed by five groups of well-trained laboratory persons. The determination of laboratory parameters (*i.e.* COD, pH, Alkalinity, and TVA) was performed by applying the standard method. The results from each laboratory showed some clearly significant differences. These results were then statistically analysed. The standard deviations obtained from the results of these laboratory groups were quite high. The discussion was made in order to find the ways in which the standard deviations can be decreased, and the resulting values of the parameters can fall within an acceptable range. The main factor affecting the results of wastewater analysis is sample collection including sampling method and preservation technique. The ways to solve this problem is to set quality control in wastewater analysis by following "Good Laboratory Practice" or GLP. and using Standard Method for the Examination of Water and Wastewater. After following the above guides, the parameter values from each laboratory became closer, the resulting standard deviation decreased and the resulting values of the parameters fall within an acceptable range with internal standard used. (Bull Chiang Mai AMS 1996; 29:72-76)

Keywords : Wastewater, total volatile acid, chemical oxygen demand, quality control

---

*Pilot Plant Development & Training Institute, King Mongkut's Institute of Technology Thonburi, Bangkok, Thailand

INTRODUCTION

The ability of accurately measure of various parameters such as pH, Alkalinity, Total volatile acid (TVA) and Chemical oxygen demand (COD); presented in wastewater is an important factor for monitoring the performance of wastewater treatment system[1].

Wastewater analysis is subjected to wide variation and reflects individual handling procedures[2]. Analysis of wastewater by different laboratory group will result in different efficiencies of recovery even using the standard method[3]. To improve the efficiency and reliable results in wastewater analysis, five groups of well-trained laboratory persons are involved. Each laboratory agreed to sampling and test samples as if they were routine. Results were analysed, discussed by the group and then compared to the others. The results from each laboratory showed clearly

significant difference with high standard deviation. The discussion was made to find the factors affecting on the test results. In this paper the factors affected the results have been studied. These included sample collection and laboratory procedures (4,5).

The study indicated that sample collection was the main factor as shown in Table 1. The problem is how to analyse the laboratory parameters of wastewater effectively. The lack of quality control in wastewater has been discussed. The objective of this study is to improve accuracy, efficiency and reliable results by using quality control in wastewater analysis.

Table 1 Effects of various factors affecting on water analysis. (1 = Series 1 = Sample collection step, 2 = Series 2 = Laboratory procedure step)

Item	pH		Alkalinity (mg/L)		TVA (mg/L)		COD (mg/L)	
	1	2	1	2	1	2	1	2
1	6.8	6.61	998	941	175	163	3,400	3,500
2	7.05	6.82	1,072	1,025	100	100	2,700	4,000
3	7.18	6.83	1,373	1,036	100	100	10,000	3,000
4	7.07	7.21	732	1,292	75	128	2,400	5,000
5	7.1	6.63	1,284	983	118	105	12,000	3,800
Mean	7.04	6.86	1,085.80	1,055.40	115.6	119.2	6,100	3,860
SD	0.14	0.22	225.45	137.49	36.94	27.09	4,543	740

MATERIALS AND METHODS

Quality Control Program

Five groups of well trained laboratory persons have been involved. All conducted quality control in wastewater analysis under. "Good Laboratory Practice" or GLP(5) as shown in Table 2

Table 2 "Good Laboratory Practice"

1. Staff and Education
2. Laboratory Management
3. Economy
4. Method Descriptions and Operation Manuals
5. Apparatus, Utensils and Reagents
6. Practical Work : Practice for Specimen Collection and Analytical Procedures
7. Report
8. Laboratory Safety

Sample collection

Wastewater samples were collected by each group from one of tapioca factory's wastewater treatment plant in Thailand. All samples (1L) were collected in 1.5L plastic bottles following

Standard Method 1992 (6). pH was analysed immediately on-site using pH meter. Samples for COD analysis were divided form sample bottles to 300 ml glass bottles, and H2SO4 was added until pH < 2. All samples were placed on ice, return to the laboratory within 2 hours and analysed immediatly within each laboratory.

Analytical methods

pH, Alkalinity, TVA and COD determinations were carried out by following Standard Method for the Examination of Water and Wastewater 1992(7). pH was analysed by potentiometric method. Alkalinity and TVA were measured by potentiometric titration method. COD was analysed by dichromate open reflux method.

Working program

To reach the objective above mentioned the following operational methodolgy was used. Wastewater analysis was performed by five groups of laboratory persons. Each laboratory have conducted routine tests under " Good Laboratory Practice" or GLP. Wastewater samples were collected and analysed by each laboratory using the same technique as mentioned above. Results were analysed and then compared to the others.

RESULTS AND DISCUSSION

Factors affecting on wastewater analysis

Table 1 shows the results of wastewater analysis obtained from five groups of well-trained laboratory persons. To evaluate the factors affecting the test results, the sample collection step was carried out by each group as if they were routine and then analysed by the same analytical method mentioned above The results and statistical analysis were shown in series 1 on Table 1. Laboratory Procedures step was shown in series2, each group collected the sample by using Standard Method mentioned above .The samples were analysed by the method as if they were routine. The results in series 1 showed wide variation and high standard deviation (pH = 7.04 ± 0.14. Alkalinity = 1085.80 ± 255.45, TVA = 115.60 ± 36.94, COD = 6100 ± 4543.13) when compared with series 2 (pH = 6.86 ± 0.22, Alkalinity = 1055.40 ± 137.49, TVA = 119.20 ± 27.09, COD = 3860 ± 740) That showed the main factor which caused high variation in the test results was sample collection step. Previous report pointed out that factors contributing to the variability in results of chemical analysis include sample handling procedures (3,8) stated that sample cellection is the most important step in environmental analytical chemistry. To solve the problem and to improve efficiency and reliable results, we introduced quality control as a tool for controlling the quality of the test results to meet the need of users and to ensure the users who want to use the reliable results (9,10).

Quality control in wastewater analysis

Table 3 shows the results of wastewater analysis obtained from five laboratory's groups. Working program was performed 3 times as shown in Day 1, Day 2, Day 3. The results from each laboratory became closer and standard deviation decreased (Day 1: pH = 6.55 ± 0.11, Alkalinity = 1590 ± 54.77, TVA = 1100 ± 187.08, COD = 3880 ± 356.37); (Day 2: pH = 6.35 ± 0.08, Alkalinity =

1216 ± 49.80, TVA = 870 ± 101.73, COD = 3500 ± 452.77); (Day 3: pH = 6.60 ± 0.05, Alkalinity = 1700 ± 0.00, TVA = 940 ± 54.77, COD = 3880 ± 130.38)

Table 4 shows the results of internal standard used in wastewater analysis from one of laboratory' s group involved in working program, fall within an acceptable range.

The results of this study suggest that quality control in wastewater analysis improves the quality of the test results. To obtain the high quality of the test results, reference materials or calibration materials may be used. The high prices of such commercially reference materials should be considered (3,5).

Table 3 Wastewater analysis from five laboratory groups after performing under "Good Laboratory Practice" (D1 = day 1, D2 = day 2, D3 = day 3)

Lab. Gr. No.	pH			Alkalinity (mg/L)			TVA (mg/L)			COD (mg/L)		
	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
1	6.45	6.48	6.55	1600	1130	1700	1400	740	1000	3800	3300	3700
2	6.65	6.32	6.55	1600	1250	1700	1100	810	900	4200	3200	4000
3	6.68	6.29	6.59	1650	1250	1700	900	860	900	3300	4300	3900
4	6.58	6.34	6.64	1500	1220	1700	1000	990	1000	4100	3300	3800
5	6.44	6.31	6.65	1600	1230	1700	1100	950	950	4000	3400	4000
Mean	6.56	6.348	6.596	1590	1216	1700	1100	870	940	3880	3500	3880
SD	0.11	0.08	0.05	54.77	49.80	0.00	187.08	101.73	54.77	356.37	452.77	130.38

Table 4 Results of Internal Standard used in wastewater analysis

Internal Standard	Actual value	Acceptable range	Day 1	Day 7	Day14	Day21	Day30
pH	7.0	7.0 ± 0.1	7.0	7.0	7.0	7.0	7.0
Alkalinity, mg $CaCO_3$/L (Na_2CC_4)	500	500 ± 5	504	503	505	502	502
COD, mg/L (KHP)	500	500 ± 25	505	492	518	502	488

CONCLUSIONS

Sample collection step in wastewater analysis have been shown to be the main factor affecting the test results. How to obtain effectively and reliable results were discussed. Quality control for laboratory has been designed to monitor testing laboratories, analytical techniques and their results. To achieve the objective of the study, "Good Laboratory Practice" has been performed, resulting in improved and reliable results on wastewater analysis.

REFERENCES

1. Amoan R and Kenedy KJ. Effect of Reactor Height on Mixing Characteristics and Performance of the Anaerobic Downflow Stationary Fixed-Film (DSFF) Reactor. J Biotech 1985; 2 : 95-106.

2. Hertz H : Accuracy and Quality Assurance in the Analytical Laboratory. ALMA Bull 1992, 10 (3): 5.
3. Kaur B and Clegg P. Testing the Waters WWT 58-62, 1994.
4. Lucchesi CA. Forces Impacting Future Chemical Analysis Enterprises. Inter Lab Pacific Rim Ed 1995; 28-31.
5. Moy D. Laboratory Analysis is Complex Work, not to be taken lighty. WM&E 1994; 5 : 12.
6. Nadkarni RA. The Quest for Quality in the Laboratory. Anal Chem 1994; 63(13) : 675 A-682A.
7. Poppiti J. Performance-Based and Prescriptive Measurement Approaches. Envi Sci Tech 1994; 28 (3) : 151A-152A.
8. Standard Methods for the Examination of Water and Wastewater 18th ed., Am Public Health Assoc Washingtcn D.C. 1992.
9. Taylor JK. Quality Assurance of Chemical Measurements. Lewis Publishers : Chelsea, MI, 1988.
10. Uldall A. Quality Assurance within Chemical Laboratory : a brief review emphasizing "Good Laboratory Practice" Scan J Clin Lab Invest 1987; 47 : 507-518.