Urinary copper/zinc ratio: a promising parameter for replacement of 24-hour urinary copper excretion for diagnosis of Wilson's disease in children

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Original article

Background: **Although 24-hour urinary copper excretion is valuable for diagnosis of Wilson's disease, accurate, timed collection entails practical difficulties. This study aimed to investigate the feasibility of morning urinary copper/creatinine or copper/zinc ratio as replacement parameter for diagnosing Wilson's disease.**

Methods: **Five random urinary samples collected during 24 hours from two inpatients were used to estimate the consistency of urinary copper/creatinine and copper/zinc ratios. The correlation of the ratios with 24-hour urinary copper excretion was studied in 15 patients with liver diseases. The diagnostic value of morning urinary copper/zinc ratio was further studied in 9 children with Wilson's disease and 22 children with other liver diseases.**

Results: **The coefficients of variation of urinary copper/creatinine and copper/zinc ratios during 24 hours were 12.5% and 9.3% respectively. The morning urinary copper/creatinine ratio, copper/zinc ratio, and 24-hour urinary copper excretion were correlated well. The area under receiver-operating characteristic curve was comparable between the morning urinary copper/zinc ratio and 24-hour urinary copper excretion (0.983 vs. 0.977).**

Conclusion: **Morning urinary copper/zinc ratio seems to be a promising parameter in replacement of 24-hour urinary copper excretion for diagnosis of Wilson's disease.**

World J Pediatr 2010;6(2):148-153

Key words: **children;**
urinary copper/zinc ratio;
Wilson's disease;
24-hour urinary copper excretion

Introduction

Wilson's disease (WD), is an autosomal recessive disorder of copper metabolism caused by mutations within the *ATP7B* gene. This causes impaired biliary copper excretion, resulting in hepatic copper toxicity and subsequent multisystem disease involving the liver, brain, cornea, skeleton and rarely the heart.[1,2] It affects 1 in 30 000 people with fatal outcome if untreated. Early diagnosis is essential because specific treatment can prevent further liver injury and neurological complications in most cases, whereas those with very severe damage require early liver transplantation to prevent a fatal outcome.[3-5] In most adults or older children with clinical evidence of liver involvement only, the diagnosis of WD may be made easily when Kayser-Fleischer (K-F) rings and low ceruloplasmin levels are present. However, in a number of cases, particularly in the younger pediatric population in whom K-F rings are frequently absent, the diagnosis of WD is most challenging.[5,6]

Although more recently our armamentarium for the diagnosis of WD has expanded to include *de-novo* molecular test for disease specific *ATP7B* mutations along with haplotype analysis for siblings of probands, this test is not universally available to all patients at this time.[7] Even if molecular test was universally available, a recent study showed that disease-causing mutations were detected in both chromosomes for *ATP7B* in only 57% of individuals,[8] thus clinicians still have to make the diagnosis according to standard clinical and laboratory criteria in most circumstances.

Although quantitative hepatic copper determination has been accepted as the "gold" criterion for the diagnosis of WD by most physicians, a value below 250 μg/g does not exclude the diagnosis.[9] In addition, there

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doi:10.1007/s12519-010-0023-4

are difficulties in acquiring enough samples for liver copper quantification, and before widespread use of transjugular liver biopsies, coagulopathy often preclude the performance of percutaneous biopsy. This leads to a reliance on urinary copper excretion and promotion of stimulation test with copper chelator *d*-penicillamine as a non-invasive method for WD diagnosis.[7,10] The quantification of 24-hour urinary copper excretion requires accurately timed urinary sample collection. However, this procedure may be associated with practical difficulties in young children, often with incomplete urinary sampling or timing errors, which may result in a wrong estimate of 24-hour urinary copper excretion. On the other hand, unexpected contaminations may occur during the long collecting process, and preservation of 24-hour urine may be problematic in hot seasons.

Based on the studies that morning or random urinary protein/creatinine ratio correlates well with timed protein excretion both in adults and in children[11-14] and that urinary protein/creatinine ratio has been widely used as a replacement parameter of 24-hour urinary protein excretion in pediatric clinics, a hypothesis was proposed that a copper ratio could be used as a replacement of 24-hour urinary copper excretion for the diagnosis of WD. The results of our preliminary studies show that morning urinary copper/zinc (Cu/Zn) ratio seems to be a promising parameter in replacement of the quantification of 24-hour urinary copper excretion.

Methods

The study protocol conformed to the ethical guidelines of the *1975 Declaration of Helsinki* and was approved by the Ethics Committee of Children's Hospital of Fudan University. Informed consent was obtained from each patient or his/her parents participating in the study and they were assured about the privacy of the data. The parents and the patients, if appropriate, were also trained in advance for correct collection of urinary samples. The patients who had not followed the 24-hour urinary collection method or had received *d*-penicillamine and/or zinc treatment within one month before their hospitalization were excluded. No special requirements for food were needed for study purpose.

Consistency study of urinary copper ratios

Two inpatients hospitalized for other than liver-related diseases were randomly selected and five random urinary samples were collected from each of them during 24 hours to estimate the consistency of urinary copper/creatinine (Cu/Cr) ratio and urinary Cu/Zn ratio.

Correlation study of copper ratios and 24-hour urinary copper excretion

Morning and 24-hour urinary samples were collected from 15 consecutive patients with liver diseases (2 with untreated WD and 13 with other liver diseases) above the age of 3 years to estimate the correlation between the morning Cu/Cr or Cu/Zn ratio and 24-hour urinary copper excretion.

Comparison of morning urinary Cu/Zn ratio with 24-hour urinary copper excretion

The morning and 24-hour urinary samples were collected from 31 consecutive patients, 22 males and 9 females, who were hospitalized between July 2005 and April 2006 for liver diseases. The mean age of the 31 patients on admission was 8.8 years with a standard derivation (SD) of 3.7 years (range, 3-16 years). Of the 31 patients, 9 were diagnosed as having WD with a mean age of 10.1±2.8 years (Table, case 1-9). Fourteen patients were easily differentiated from WD diagnosis, including 6 patients with chronic hepatitis B virus infection who were enrolled for liver biopsy, 2 patients with Epstein-Barr virus infection, 1 patient with acute hepatitis A, 1 patient with glycogen storage disease type 1, 1 patient with Budd-Chiari syndrome, 1 patient with parasite infection (pentastomidosis), 1 patient with Duchenne muscular dystrophy, and 1 patient with tumor. Eight non-Wilson patients in whom only a clinically suspected diagnosis was made or the clinical situation might be easily confused with WD and 9 WD patients were reviewed in terms of WD diagnostic scores[15] (Table).

All WD patients except patient 9 had a WD score ≥4. One patient was clarified into N1 and 8 were classified into H2. All of them had an extremely low ceruloplasmin level (<0.08 g/L), and K-F rings were found in 5 patients with age ranging from 8 years to 13 years. In the rest 4 patients whose K-F rings were absent, 3 demonstrated a high excretion of 24-hour urinary copper, and 1 only a slightly high excretion of 24-hour urinary copper (patient 9) and this patient also showed an extremely low level of serum copper (less than one eighth of the lower limit of normal range). After 6 months of penicillamine treatment, the liver function of patient 9 normalized, but the extremely low level of ceruloplasmin persisted. The diagnosis of WD was made in considering the persistent extremely low serum ceruloplasmin level and good response to WD treatment. Patient 5 received penicillinamine one month before admission. Other patients received no treatment with penicillinamine or zinc for liver diseases.

All the other 8 patients had a WD diagnostic score less than 2 which made the WD very unlikely.

Liver failure was diagnosed in 3 patients, and 2 (patients 16 and 17) of them were probably caused by hemophagocytic lymphohistiocytosis (HLH) according to typical clinical manifestations and pathological findings of hemophagocytosis in bone marrow. The levels of ceruloplasmin were marginal at beginning, but normalized during the follow up (0.170-0.381 in patient 16; 0.209-0.351 and then 0.529 in patient 17); the condition of the patients deteriorated and they died eventually. The third patient who had liver failure (patient 12) was caused by congenital heart disease and heart failure, and his condition improved after the improvement of heart function. His WD score was only 1. Two patients were clinically diagnosed as having acute viral hepatitis other than hepatitis virus A-E. Of them, one possibly suffered from herpes simple virus (HSV) infection indicated by positive IgM antibody, and the other suffered from enterovirus also indicated serologically. Three patients had suspected diagnosis of cytomegalovirus infection, idiopathic liver disease, and benign recurrent intrahepatic cholestasis, respectively.

Urinary sample collection and determination

The first morning urinary sample was obtained in a metal-free dispensable plastic tube, and then urine was collected for 24 hours in an acid and high purity water washed metal-free plastic bottle. The volume of 24 hours urine was determined and then 20 ml urinary sample was transferred to a metal-free dispensable plastic tube and frozen at -20°C along with morning urine sample until analysis. The remaining 24-hour urine sample was sent to a clinical laboratory for determination of 24-hour urinary copper excretion. Copper excretion more than 100 μg/24 h was regarded as one of the parameters of a comprehensive assessment for the diagnosis of WD.

Urinary copper and zinc were determined directly by flame atomic absorption spectrometry (Perkin Elmer 5000; Perkin-Elmer Cetus, Norwalk, CT). Urinary creatinine concentration was determined using the creatinase method in a chemistry analyzer (ADVIA 1650/Mega Bayer). Consistency and correlation were determined in one run, and specificity and sensitivity were determined in a second run by the same technician so as to eliminate the difference between the runs. 24-hour urinary copper excretion was expressed as μg/24 h, 50 μg/24 h was used as the normal upper limit, and more than 100 μg/24 h was regarded as one of the diagnostic parameters of WD. Cu/Cr and Cu/Zn ratios were calculated by dividing the copper concentration (μg/ml) with creatinine concentration (mmol/ml) or zinc concentration (μg/ml), respectively.

Table. Clinical information of patients with Wilson's disease (patients 1-9) and patients with other liver diseases who need differentiation (patients 10-17)

Case no.	Sex/ age	WD scores	Diagnosis/ subtype	TB/DB	ALT/ AST	PT	Alb	CP	24-hour urinary copper excretion (mg)	K-F rings	Brain damage	Coombs negative hemolysis	Biopsy	
													Features	Rhodamin stain
1	F/13	8	WD/N1	6.1/2.5	68.35	15.2	49.4	<0.08	1180.5	+	+	-	NA	NA
2	M/8	6	WD/H2	15.4/10.3	54/81	19.8	NA	<0.08	353.9	+	-	-	NA	NA
3	M/10	5	WD/H2	18.1/7.9	38/42	NA	33.8	<0.08	181.3	+	-	-	NA	NA
4	F/9	5	WD/H2	10.9/2.7	26/33	14.7	43.4	0.023	57.2	+	-	-	NA	NA
5	M/10	5	WD/H2	11.1/3.8	136/124	NA	35.6	<0.08	57	+	-	-	NA	NA
6	M/7	4	WD/H2	6.2/1.7	95/59	13.5	NA	<0.08	283.4	-	-	-	NA	NA
7	M/10	4	WD/H2	N/N	46/51	13.5	43.0	0.02	157.9	-	-	-	Steatosis/mild fibrosis	-
8	F/5	4	WD/H2	8.9/3.2	256/111	12.8	44.9	0.08	355	-	-	-	NA	NA
9	F/11	3	WD/H2	13.9/3.8	83/42	14.2	37.5	<0.08	52.6	-	-	-	Steatosis/cirrhosis	-
10	M/15	2 (1)*	Acute hepatitis	12.1/5.5	47/30	13.4	NA	0.167 (0.304)*	51.3	-	-	-	NA	NA
11	M/7	1	CMV?	5.5/2.2	90/101	13.9	45	0.228	26	-	-	-	Normal	+
12	M/15	1	Liver failure	164/52.8	2314/1661	14.8	27	0.166	43.9	-	-	-	NA	NA
13	M/8	1	Acute hepatitis	8.5/2.1	95/41	15.4	51.2	0.302	55.2	-	-	-	Mild nonspecific inflammation	-
14	M/8	0	BRIC?	141.5/112.4	22/53	22.6	44.5	0.551	19.4	-	-	-	Severe cholestasis	-
15	M/5	0	Idiopathic hepatitis?	6.1/1.9	197/122	12.7	49.7	NA	16.8	-	-	-	Mild inflammation	-
16	M/11	2 (1)*	Liver failure	491.6/347.8	387/178	32.9	31.9	0.170 (0.381)*	96.8	-	-	NA	NA	NA
17	M/10	1	Liver failure	376/296	30/46	17.7	31.8	0.209	52.8	-	-	-	Steatosis/inflammation/ bile duct proliferation	-

*: numbers in parentheses are values at follow-up. TB: serum total bilirubin, normal <17.1 mmol/L; DB: serum direct bilirubin, normal <7.0 mmol/L; ALT: alanine aminotransferase, normal <40 U/L; AST: aspartate aminotransferase, normal <40 U/L; PT: prothrombin time, normal <15s; Alb: albumin, normal 35-55 g/L; CP: serum ceruloplasmin, normal >0.2 g/L; 24-hour urinary copper excretion, normal <50 μg; NA: not available.

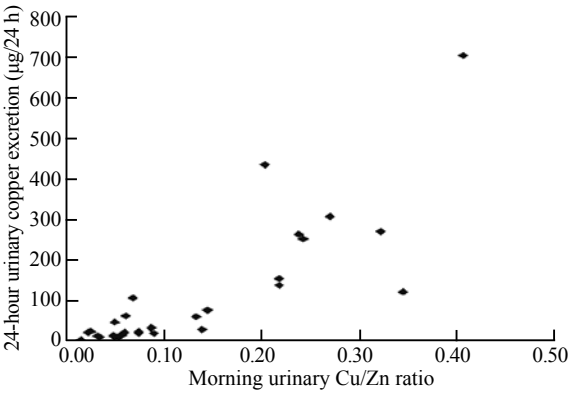

Fig. 1. The correlation of morning urinary copper/zinc ratio (Cu/Zn) with 24-hour urinary copper excretion (r=0.826, P<0.001). Cu/Zn was calculated by dividing the morning urine copper concentration (μg/ml) with morning urine zinc concentration (μg/ml).

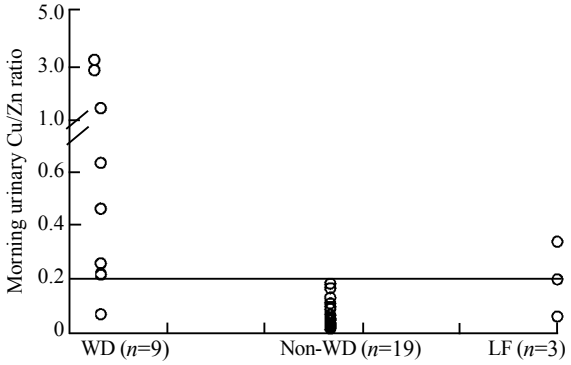

Fig. 2. The scatterplot of morning urinary Cu/Zn ratio in different groups of patients. Cu/Zn ratio was calculated by dividing the morning urinary copper concentration (μg/ml) with morning urinary zinc concentration (μg/ml). WD: Wilson's disease patients; Non-WD: patients with liver disease other than WD or liver failure; LF: liver failure patients.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used for demographic and baseline data and presented as mean/median or percentage. To evaluate the correlation between the morning urinary Cu/Cr or Cu/Zn ratio and 24-hour urinary protein excretion, simple linear regression with calculation of Pearson's correlation coefficient (r) was used. The accuracy of a morning urinary Cu/Zn ratio for detection of WD was compared with the 24-hour urinary copper excretion by comprehensive clinical diagnosis as a "gold" standard.

Results

The consistency of urinary copper ratios

The mean Cu/Cr ratio of 5 random urinary samples during 24 hours was 0.102±0.008 and 0.099±0.017 respectively in the 2 inpatients, which created a coefficient of variation (CV) 7.8% and 17.2%, respectively, with a mean of 12.5%. The mean Cu/Zn ratio was 0.082±0.011 and 0.081±0.004 respectively, which produced a CV of 13.4% and 4.9% respectively with a mean of 9.3%.

The correlation of copper ratios and 24-hour urinary excretion

Significant correlation was found between 24-hour urinary copper excretion and Cu/Cr ratio or Cu/Zn ratio in 2 WD children and 13 children with other liver diseases. A slightly better correlation was found between 24-hour urinary excretion and Cu/Zn ratio than between 24-hour urinary excretion and Cu/Cr ratio (Cu/Zn, r=0.891, t=7.077, P<0.001 vs. Cu/Cr, r=0.767, t=4.314, P<0.001). The mean Cu/Zn ratio was 0.285±0.229 for the 2 WD children, and 0.076±0.031 for the 13 children with other liver diseases. Their mean Cu/Cr ratio was 0.408±0.401 and 0.135±0.060 respectively. The mean Cu/Zn ratio was slightly higher than the mean Cu/Cr ratio (3.8 vs. 3.0). Therefore, the Cu/Zn ratio was chosen as a parameter for further investigation and a strong correlation between the morning urinary Cu/Zn ratio and 24-hour urinary copper excretion was further demonstrated (Fig. 1).

The diagnostic value of morning urinary Cu/Zn ratio

The sensitivity and specificity of morning urinary Cu/Zn ratio in diagnosing WD were compared with those of 24-hour urinary copper excretion (Fig. 2). The area under ROC with 95% confidence interval of 24-hour urinary copper excretion, morning urinary Cu/Zn ratio, and Cu/Zn ratio measured using 24-hour urine sample was 0.977 (0.935-1.000), 0.983 (0.946-1.000), and 0.989 (0.962-1.000), respectively. No significant difference was found between them (χ^2=0.45, P=0.799). With 0.2 as a cutoff value, morning urinary Cu/Zn ratio produced a sensitivity of 88.9% and a specificity of 90.9%. If the 3 patients with liver failure and the WD patient who had received penicillinamine one month before admission (patient 5) were excluded, both the sensitivity and specificity reached 100% (Fig. 2).

Discussion

WD as a treatable genetic disorder has a benign course if diagnosis is made at an early stage.[16] In childhood, it is evidenced by liver damage that can mimic the whole spectrum of parenchymal liver diseases. The diagnosis

of WD may be problematic because K-F rings are not seen in most pediatric patients and ceruloplasmin levels may be normal in some WD patients and low in some carriers.[16,17] Many studies have emphasized the diagnostic value of 24-hour urinary copper excretion in distinguishing WD from other liver diseases.[7,10,18] However, it is relatively cumbersome and time-consuming, and could be inaccurate because of incomplete collection.[11] Therefore, we investigated the feasibility of replacing the 24-hour urinary copper excretion with morning urinary copper ratio.

We investigated two urinary copper ratios, morning Cu/Cr ratio and Cu/Zn ratio. Cu/Cr ratio was chosen as a candidate because creatinine is excreted in a steady speed and not re-absorbed in the course of urinary formation, and therefore a suitable parameter was used to correct the effect of urinary condensation or dilution.[19] Some parameters that are based on creatinine correction, such as random or morning protein/creatinine ratio, calcium/creatinine ratio, and others that are highly correlated with the parameters based on 24-hour urinary collection are widely accepted as replacement parameters in related fields.[11-14,19] Cu/Zn ratio was proposed as a candidate since copper and zinc are trace elements and possible antagonism exists between them.[20] WD patients in whom the *ATP7B* defects severely damage the excretion of copper via the hepatobiliary system show a slightly increased zinc concentration in their liver.[21] It is supposed that zinc may be a good parameter to correct urine condensation or dilution in WD patients. Cu/Zn ratio should be more favorable considering that both elements could be simultaneously determined by the same instrument.[22]

In this study, the daily consistency was acceptable for both random urinary Cu/Cr ratio and Cu/Zn ratio, and their correlation with 24-hour urinary copper excretion was statistically significant. However, urinary Cu/Zn ratio seems to behave better than Cu/Cr ratio in all terms including the parameter's daily consistency, its correlation with 24-hour urinary copper excretion and the ability to discriminate WD from other liver diseases. Therefore, further investigation should concentrate on morning urinary Cu/Zn ratio only.

A strong correlation was found between morning urinary Cu/Zn ratio and 24-hour urinary copper excretion in this study and the diagnostic value of morning urinary Cu/Zn ratio was compared with 24-hour urinary copper excretion. The area under ROC curve was similar between urinary Cu/Zn ratios and 24-hour urinary copper excretion. The urinary Cu/Zn ratio, especially Cu/Zn ratio measured using 24-hour urinary samples, behaves a little bit better for the diagnosis of WD, even routine 24-hour urinary copper excretion has been used as one of the parameters in the initial diagnosis of the case series. We speculate that this may be incurred by the inaccurate collection of 24-hour urine although rigorous measures have been taken.[23] A single cutoff value for 24-hour urinary copper excretion used for a wide age group without correction may be another reason although a correction for age is believed to be not necessary.

Many clinicians use zinc supplement as an alternative or complementary treatment of WD, which may interfere the urinary Cu/Zn ratio as well as the 24-hour urinary copper excretion. However, before a definite diagnosis of WD is established, zinc is rarely prescribed for liver disease patients. Therefore, the potential impact is minimal when urinary Cu/Zn ratio is used as a diagnostic parameter. The impact of specific therapy on urinary Cu/Zn ratio needs to be carefully assessed in patients who receive zinc and/or chelator therapy because urinary Cu/Zn ratio may also serve as an alternative parameter for assessing treatment efficacy in the future.

In conclusion, morning urinary Cu/Zn ratio is a hopeful parameter to replace the determination of 24-hour urinary copper excretion for the diagnosis of WD, although a definite conclusion could not be made from this study because of the relatively small number of patients. Since morning urinary sample collection is much easier, time-saving, and cost-effective, and its results are promising, a prospective study with a large number of patients is warranted.

Funding: This paper was supported by a grant from Shanghai Public Health Key Subject Construction (08GWZX0102).
Ethical approval: The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the Children's Hospital of Fudan University.
Competing interest: None declared.
Contributors: Wang JS proposed the study and wrote the first draft. Lu Y collected the data and orgnized the determination. All authors contributed to the design and interpretation of the study and to further drafts.

References

1 Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nat Genet 1993;5:327-337.
2 Petrukhin K, Fischer SG, Pirastu M, Tanzi RE, Chernov I, Devoto M, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. Nat Genet 1993;5:338-343.
3 Ala A, Schilsky ML. Wilson disease: pathophysiology, diagnosis, treatment, and screening. Clin Liver Dis 2004;8:787-805.
4 Brewer GJ, Askari FK. Wilson's disease: clinical management and therapy. J Hepatol 2005;42:S13-S21.
5 Schilsky ML. Diagnosis and treatment of Wilson's disease.

Pediatr Transplantation 2002;6:15-19.
6 Steindl P, Ferenci P, Dienes HP, Grimm G, Pabinger I, Madl C, et al. Wilson's disease in patients presenting with liver disease: a diagnostic challenge. Gastroenterology 1997;113:212-218.
7 Schilsky ML. Non-invasive testing for Wilson disease: revisiting the *d*-penicillamine challenge test. J Hepatol 2007; 47:172-173.
8 Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut 2007;56:115-120.
9 Ferenci P, Steindl-Munda P, Vogel W, Jessner W, Gschwantler M, Stauber R, et al. Diagnostic value of quantitative hepatic copper determination in patients with Wilson's Disease. Clin Gastroenterol Hepatol 2005;3:811-818.
10 Müller T, Koppikar S, Taylor RM, Carragher F, Schlenck B, Heinz-Erian P, et al. Re-evaluation of the penicillamine challenge test in the diagnosis of Wilson's disease in children. J Hepatol 2007;47:270-276.
11 Morales JV, Weber R, Wagner MB, Barros EJ. Is morning urinary protein/creatinine ratio a reliable estimator of 24-hour proteinuria in patients with glomerulonephritis and different levels of renal function? J Nephrol 2004;17:666-672.
12 Pahwa MB, Seth S, Khosla A. Significance of urine protein/creatinine ratio in pregnancy-induced hypertension. Clin Chim Acta 2007;382:145-147.
13 Gonsales Valério E, Lopes Ramos JG, Martins-Costa SH, Müller AL. Variation in the urinary protein/creatinine ratio at four different periods of the day in hypertensive pregnant women. Hypertens Pregnancy 2005;24:213-221.
14 Kaneko K, Someya T, Nishizaki N, Shimojima T, Ohtaki R, Kaneko K. Simplified quantification of urinary protein excretion using a novel dipstick in children. Pediatr Nephrol 2005;20:834-836.
15 Ferenci P, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003;23:139-142.
16 Roberts EA, Schilsky ML. A practice Guideline on Wilson disease. Hepatology 2003;37:1475-1492.
17 Cauza E, Maier-Dobersberger T, Polli C, Kaserer K, Kramer L, Ferenci P. Screening for wilson's disease in patients with liver disease by serum ceruloplasmin. J Hepatol 1997;27:358-362.
18 Martins da Costa C, Baldwin D, Portmann B, Lolin Y, Mowat AP, Mieli-Vergani G. Value of urinary copper excretion after penicillamine challenge in the diagnosis of Wilson's disease. Hepatology 1992;15:609-615.
19 Torng S, Rigatto C, Rush DN, Nickerson P, Jeffery JR. The urine protein to creatinine ratio (P/C) as a predictor of 24-hour urine protein excretion in renal transplant patients. Transplantation 2001;72:1453-1456.
20 Pang Y, Applegate TJ. Effects of dietary copper supplementation and copper source on digesta pH, calcium, zinc, and copper complex size in the gastrointestinal tract of the broiler chicken. Poult Sci 2007;86:531-537.
21 Stamoulis I, Kouraklis G, Theocharis S. Zinc and the liver: an active interaction. Dig Dis Sci 2007;52:1595-1612.
22 Gao L, Ren S. Simultaneous spectrophotometric determination of four metals by two kinds of partial least squares methods. Spectrochim Acta A Mol Biomol Spectrosc 2005;61:3013-3019.
23 Christopher-Stine L, Petri M, Astor BC, Fine D. Urine protein-to-creatinine ratio is a reliable measure of proteinuria in lupus nephritis. J Rheumatol 2004;31:1557-1559.

Received July 3, 2008
Accepted after revision January 21, 2009