Original Article

Role of N-Acetylcysteine for Improvement of the Aminotransferase Level in Metabolic Syndrome with Persistent Liver Enzyme Elevation

Panamonta N
Chunlertrith K

ABSTRACT

Background: Insulin resistance and metabolic syndrome is strongly associated with non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). According to the pathogenesis of NASH, medications that minimize oxidative stress may prove useful. N-acetylcysteine (NAC) is one of glutathione prodrugs which could limit the production of reactive oxygen species. This study aims to investigate the effect of NAC on liver biochemical improvement in metabolic syndrome patients with persistent aminotransferase level elevation.

Methods: Fourteen patients were diagnosed with metabolic syndrome and had persistent alanine aminotransferase (ALT) level over 1.5 times the upper limit of the normal value more than 6 months, after two-month of lifestyle modification, patients were randomized into 2 groups. The first group received NAC of 600 mg orally per day plus the standard treatment for NASH (NAC group) and the second group received only the standard treatment of NASH (control group) for 8 weeks. Changes in variables were measured from baseline to the end of treatment including; serum ALT, AST, fasting serum glucose, triglyceride, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and body mass index (BMI).

Results: The baseline characteristics between two groups were similar. At the end of the 8-week treatment period, there was a significant change in the mean ALT and AST levels in the control group ($p = 0.03$ and 0.04) but not in the treatment group ($p = 0.27$ and 0.53), respectively. All of the patients tolerated the drug well, without any serious adverse effects.

Conclusion: NAC is a safe medication and can be tolerated well in patients with NAFLD, however, our study could not detect any statistically significant improvement in the level of serum aminotransferase.

Key words : N-acetylcysteine, nonalcoholic steatohepatitis, serum aminotransferase

[Thai J Gastroenterol 2010; 11(3): 149-154.]

INTRODUCTION

Insulin resistance and metabolic syndrome is strongly associated with nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH)[1-3]. The respective prevalence of NAFLD and NASH in developed countries is estimated at 20- 30% and 2- 3%[4-6]. In Thailand, the respective NAFLD and NASH in non-HBV, non-HCV chronic hepatitis is 54.3% and 76.1%[7]. There is no general consensus on the effectiveness of any therapeutic agent for the treatment of NASH other than lifestyle modification and specific medical treatment of the metabolic syndrome.

Division of Gastroenterology, Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
Address for Correspondence: *Kitti Chunlertrith, M.D., Division of Gastroenterology, Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.*

THAI J
150 GASTROENTEROL
2010

Role of N-Acetylcysteine for Improvement of the Aminotransferase Level in Metabolic Syndrome with Persistent Liver Enzyme Elevation

According to "the two-hits hypothesis" on the pathogenesis of NASH[8,9], the first hit is fat accumulation due to dysregulation of fatty acid metabolism which is associated with insulin resistance and hyperinsulinemia. The second hit, is oxidative stress and lipid peroxidation, which are associated with cellular adaptation, altered signaling pathways and some environmental and genetic factors. This might provide a basis for the use of drugs that could protect hepatocytes from oxidative stress. Medications that minimize oxidative stress such as vitamin E, betaine, superoxide dismutase, and N-acetylcysteine may be useful.

N-acetylcysteine (NAC) is a glutathione precursor which increases the glutathione level in hepatocytes and has some regulatory effects on microcirculation. Increasing the glutathione level could limit the production of reactive oxygen species which causes hepatocellular injury. Glutathione prodrugs have produced beneficial effects in virtually every known experimental model of hepatotoxicity[10]. NAC is the most well-known glutathione prodrug in clinical practice with more safety profiles and acceptable costs. Even though many experimental studies showed improvement in liver histology in rats with NASH and attenuation of oxidative stress from NAC treatment[11], until now there are few studies that demonstrate the benefit of NAC in patients with NAFLD[12,13]. The cause and effect relationships of those studies cannot be fully explained as the effect of NAC on the improvement of biochemical parameters. Furthermore, there is no clinical trial on NAC as a treatment for patients with NAFLD in Thailand. The present study aimed to investigate the effect of NAC on liver biochemical improvement in patients with NAFLD and metabolic syndrome.

MATERIALS AND METHODS

Population

The present study recruited NAFLD patients with metabolic syndrome on regular follow-up at the General Medicine Outpatient Unit of Srinagarind Hospital between January and December 2009. This study was approved by the Khon Kaen University Ethics Committee for Human Research. All patients gave informed consent before entering the study.

Inclusion criteria

1. Patients with metabolic syndrome defined as having at least three of the five of the following[14,15]:

1) Abdominal obesity waist circumference > 90 cm in men; > 80 cm in women or a BMI ≥ 25 kg/m²

2) Serum triglyceride ≥ 150 mg/dL

3) Serum HDL ≤ 40 mg/dL in men; ≤ 50 mg/dL in women

4) BP ≥ 130/85 mmHg or currently on antihypertensive therapy

5) Fasting blood sugar ≥ 110 mg/dL

2. Persistent liver enzyme ALT elevation more than 1.5 times the upper limit of the normal value on at least 2 separate occasions, 6 months apart, after strict dietary control for 2 months

3. Between 18 and 80 years of age

Exclusion criteria[16,17]

1. Alcohol abuse of more than 20 g/day in the previous 6 months

2. Serologic evidence of viral hepatitis

3. Previous evidence of other hepatitis and chronic liver disease : autoimmune hepatitis, primary biliary cirrhosis, Wilson's disease, hemochromatosis

4. Decompensated or advanced cirrhosis Child-Pugh score B or C

5. Use of any of the following medications : amiodarone, tamoxifen, methotrexate, antioxidants, metformin, glitazone, theophylline, warfarin, glucocorticoids, estrogen or progesterone therapy

6. Previous surgical procedures : gastric bypass, jejunoileal bypass, extensive small bowel resection

7. Other co-morbid diseases : congestive heart failure, end stage renal disease, pregnancy, HIV infection

Run-in period

Studied patients required to be the standard treatment for NASH by under a controlled diet for at least 2 months , and given instructions in controlling weight, exercise counseling, and counseling on cessation of smoking, herbal medication, vitamin or mineral supplements. The recommendations included: (1) a weight reduction at least 10% from baseline body weight; (2) moderate exercise for at least 30 minutes duration, 5 days a week; (3) a diet including complex carbohydrates and fiber (comprising at least 50% of total energy) and fat 30% of total energy with unsaturated fatty acids being the major component. All diabetic patients were treated with oral antidiabetic drugs (not

Panamonta N, Chunlertrith K

metformin and glitziness). Hypertensive and hyperlipidemia patients were treated with oral antihypertensive agents and oral lipid lowering drugs, respectively.

Treatment period

After receiving the standard diet and exercise control program, patients with an ALT persistently > 1.5 times the upper limit of the normal value (30 U/L in men and 19 U/L in women) on at least two occasions six months apart were enrolled in the study. Enrolled patients were randomly assigned to either the experimental or the control group. Both groups received the standard medical treatment for metabolic syndrome and NAFLD. Only the experimental group received NAC 600 mg/day for a period of 8 weeks. At baseline and completion of the 8-week treatment period, the following variables were measured: serum ALT, AST, fasting serum glucose, triglyceride, LDL, HDL, total bilirubin, albumin, globulin, anti-HIV, HBsAg, Anti-HCV, ANA, BMI, waist-hip ratio (WHR), Homeostasis Model Assessment of insulin resistance index [$HOMA_{iR}$ = fasting serum insulin (µIU/mL) × fasting serum glucose (mmol/L) / 22.5]. During the NAC treatment, patients were follow-up every 4-weeks. Serum ALT, AST, fasting serum glucose, triglyceride, LDL, HDL, and BMI were measured on each visit. Figure 1 shows the conceptual framework of the study.

Outcome measurement

The study outcome aimed to compare ALT levels between the group of receiving NAC treatment versus the control group (decreased >50% from baseline) or ALT normalization. Changes in the ALT and AST level as well as other metabolic parameters (i.e. fasting serum glucose, LDL, HDL, triglyceride, body weight) were compared between the groups.
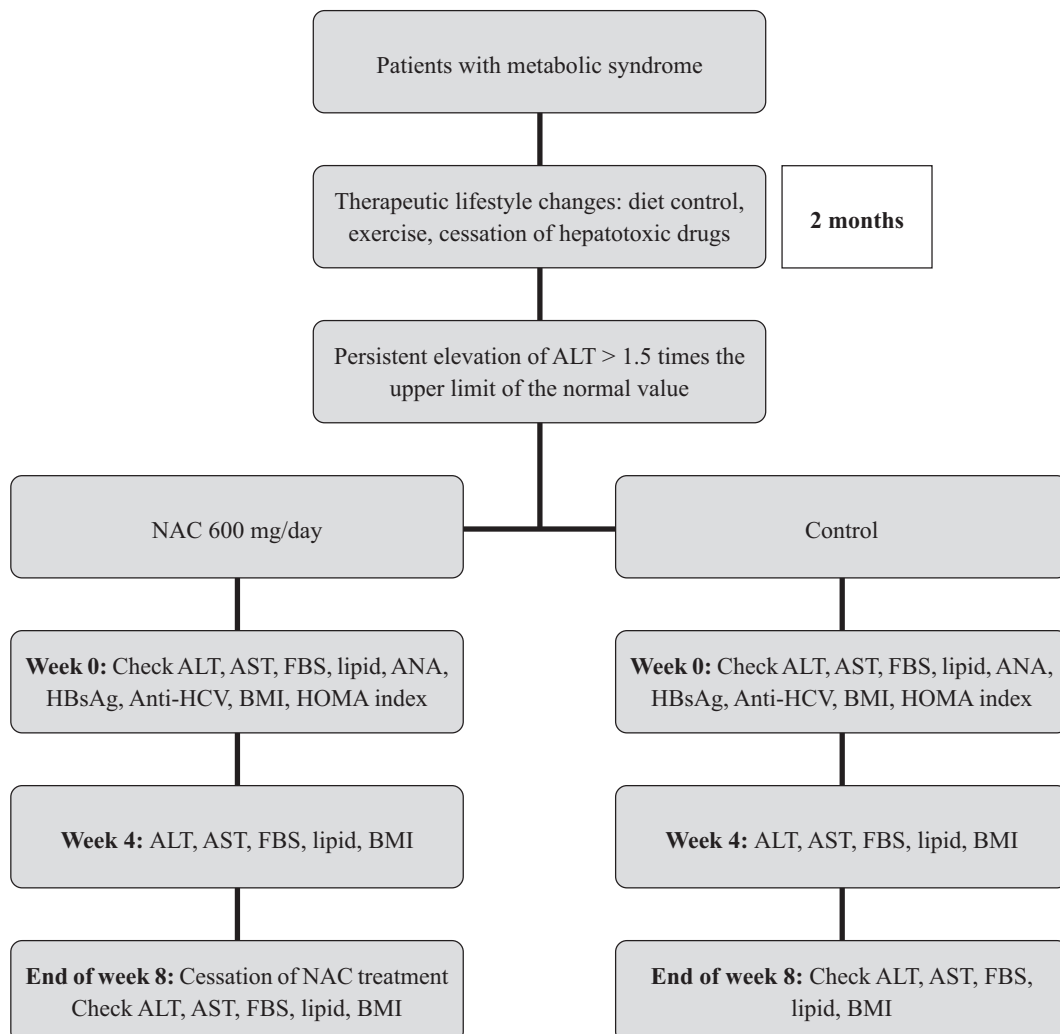

Figure 1. Conceptual framework of the study.

152 **THAI J**
GASTROENTEROL
2010

Role of N-Acetylcysteine for Improvement of the Aminotransferase Level in
Metabolic Syndrome with Persistent Liver Enzyme Elevation

Statistical analysis

Changes in the ALT, AST, fasting serum glucose, triglyceride, LDL, HDL, and BMI after the 8-week treatment period were analyzed for mean ± SD or median ± IQR, then compared using the two-sided *t* test between those on NAC treatment vs. those on placebo. Changes in the ALT and AST at the end of treatment were presented as a percentage of change from the baseline and compared between groups using the two-sided *t* test. The *t*-test was used to compare the continuous data (i.e. for age, BMI, fasting serum glucose) and the Chi-square (or Fisher's exact test) for categorical numbers (i.e. sex). A *p*-value of less than 0.05 was accepted as statistically significant.

RESULTS

A total of 72 patients with metabolic syndrome were included in the study. Fifty-two patients were excluded for the following reasons: unable to join the regular follow-up program (n = 23), serum ALT level was decreased to less than 1.5 times the upper limit of the normal value after a 2-month period of lifestyle modification (n = 20), unable to complete the lifestyle modification program which included failure to stop alcohol drinking (n = 3), previously received metformin as a treatment for diabetes mellitus (n = 3), positive for HBsAg (n = 2), anti-HCV positive (n = 1), and ANA positivity (titer ≥ 1:80) (n = 6). Fourteen patients were successfully included. The baseline characteristics are presented in Table 1. There was no statistically significant difference in sex distribution between groups, albeit the treatment group had more males than females (*p* = 0.12). The other baseline characteristics were similar between groups. The baseline liver biochemical parameters are presented in Table 2. The baseline serum ALT level was slightly higher in the NAC group than in the control group (*p* = 0.29).

Table 1. Baseline characteristics of the patients.

Parameters Number (%); Mean ± SD	NAC group (N = 8)	Control group (N = 6)	*p*-value
Gender (male/female)	7/1	3/3	0.12
Age (years)	50.1 ± 6.5	49.5 ± 7.6	0.87
BMI (kg/m^2)	27.6 ± 3.2	29.2 ± 1.1	0.26
WHR	0.9 ± 0.05	0.9 ± 0.05	0.82
Fasting serum glucose (mg/dL)	115.6 ± 27.4	113.3 ± 31.9	0.89
Systolic blood pressure (mmHg)	134 ± 16.2	126.7 ± 13.7	0.39
Diastolic blood pressure (mmHg)	84.1 ± 13.5	78.3 ± 9.8	0.39
LDL-cholesterol (mg/dL)	146.7 ± 29.8	144.2 ± 46.3	0.90
HDL-cholesterol (mg/dL)	44.1 ± 6.9	47 ± 11.2	0.56
Triglyceride (mg/dL)	179 ± 84.9	154.3 ± 32.2	0.52
Serum insulin (µIU/mL)	32.0 ± 23.9	35.4 ± 23.1	0.81
HOMA$_{iR}$ index	9.0 ± 7.8	10.7 ± 8.7	0.73

Abbreviations: BMI, body mass index; LDL-cholesterol, cholesterol bound to low-density lipoproteins; HDL-cholesterol, cholesterol bound to high-density lipoproteins; HOMA$_{iR}$, homeostasis model assessment of insulin resistance

Table 2. Baseline liver biochemical parameters.

Parameters Mean ± SD	NAC group (N = 8)	Control group (N = 6)	*p*-value
ALT (U/L)	80.3 ± 21.1	64.3 ± 32.9	0.29
AST (U/L)	46.3 ± 16.0	45 ± 26.6	0.91
ALP (U/L)	78.6 ± 19.7	82.2 ± 26.9	0.78
Total bilirubin (mg/dL)	0.8 ± 0.3	0.7 ± 0.3	0.68
Albumin (g/L)	4.7 ± 0.2	4.7 ± 0.3	0.72

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase

Table 3. Mean changes in liver biochemical and metabolic parameters at the end of the 4-week NAC treatment period.

Parameters (Mean ± SD)	NAC group (N = 8)			Control group (N = 6)			*p*-value*
	Baseline	Mean change at 4-week	*p* value	Baseline	Mean change at 4-week	*p* value	
ALT (U/L)	80.3 ± 21.1	-12.9 ± 24.3	0.18	64.3 ± 32.9	-6.8 ± 15.3	0.33	0.60
AST (U/L)	46.3 ± 16.0	-0.6 ± 16.2	0.92	45.0 ± 26.6	-7.5 ± 11.5	0.17	0.39
FBS (mg/dL)	115.6 ± 27.4	8.5 ± 14.9	0.22	113.3 ± 31.9	-0.7 ± 16.3	0.95	0.42
BMI (kg/m^2)	27.6 ± 3.2	0.3 ± 0.7	0.29	29.2 ± 1.1	0.3 ± 0.5	0.18	0.95
LDL (mg/dL)	146.8 ± 29.8	6.5 ± 12.1	0.17	144.2 ± 46.3	0.2 ± 38.9	0.99	0.67
Triglyceride (mg/dL)	179 ± 84.9	-31.9 ± 49.4	0.11	154.3 ± 32.2	0.8 ± 63.0	0.98	0.32
HDL (mg/dL)	44.1 ± 6.9	3.4 ± 5.5	0.13	47 ± 11.2	-5.2 ± 18.4	0.50	0.84

*Difference of mean change in parameters at the end of 4-week treatment period of both groups
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; BMI, body mass index; LDL-cholesterol, cholesterol bound to low-density lipoproteins; HDL-cholesterol, cholesterol bound to high-density lipoproteins

Table 4. Mean changes in liver biochemical and metabolic parameters at the end of the 8-week NAC treatment period.

Parameters (Mean ± SD)	NAC group (N = 8)			Control group (N = 6)			*p*-value**
	Baseline	Mean change at 4-week	*p* value	Baseline	Mean change at 4-week	*p* value	
ALT (U/L)	80.3 ± 21.1	-9.3 ± 21.9	0.27	64.3 ± 32.9	-17.2 ± 13.5	0.03*	0.45
AST (U/L)	46.3 ± 16.0	-2.6 ± 11.3	0.53	45.0 ± 26.6	-11.3 ± 9.8	0.04*	
FBS (mg/dL)	115.6 ± 27.4	3.0 ± 16.870	0.68	113.3 ± 31.9	-17.5 ± 42.7	0.36	0.30
BMI (kg/m^2)	27.6 ± 3.2	0.1 ± 0.7	0.65	29.2 ± 1.1	0.1 ± 0.7	0.71	0.98
LDL (mg/dL)	146.8 ± 29.8	-14.0 ± 36.2	0.31	144.2 ± 46.3	-5.7 ± 47.6	0.78	0.72
Triglyceride (mg/dL)	179 ± 84.9	0.9 ± 65.6	0.97	154.3 ± 32.2	-7.7 ± 91.7	0.85	0.84
HDL (mg/dL)	44.1 ± 6.9	1.9 ± 5.9	0.39	47 ± 11.2	-1.0 ± 5.2	0.66	0.36

*p <0.05
**Difference of mean change in parameters at the end of 4-week treatment period of both groups
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; BMI, body mass index; LDL-cholesterol, cholesterol bound to low-density lipoproteins; HDL-cholesterol, cholesterol bound to high-density lipoproteins

At the end of 4-week period of treatment, there was no statistically significant change in the liver biochemical and other metabolic parameters within or between groups (Table 3). At the end of the 8-week treatment period, there was a significant change in the mean ALT and AST levels in the control group (p = 0.03 and 0.04, respectively) (Table 4). There was no significant difference in the changes of aminotransferase between both groups at the end of treatment. The percentage of change in the ALT and AST at the end of treatment was slightly greater in the control group than those in NAC group (p = 0.29 and 0.16) (Table 5). Only one patient in the control group presented a greater than 50% decreasing in ALT level from baseline at the end of treatment. All of the patients (100%) in the NAC group tolerated the drug well without any serious adverse events.

Table 5. Mean changes in liver biochemical parameters at the end of treatment; data shown in percent of changes from baseline.

Parameters	NAC group (N = 8)	Control group (N = 6)	*p*-value
ALT	-11.5%	-26.7%	0.29
AST	-5.7%	-25.2%	0.16

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase

DISCUSSION

This is the first randomized controlled trial in Thailand to investigate the effects of NAC on the improvements of liver biochemical parameters in patients with NAFLD and metabolic syndrome. Liver biopsy

THAI J
154 **Gastroenterol**
2010

Role of N-Acetylcysteine for Improvement of the Aminotransferase Level in
Metabolic Syndrome with Persistent Liver Enzyme Elevation

was not performed in the present study because it was agreed that the risks to patients outweighed any benefits. All of patients with NAFLD were diagnosed by ultrasonography of upper abdomen.

Even though NAC appears to improve the aminotransferase level according to the pathogenesis of NASH, the present study failed to detect any statistically significant change in the NAC group. This could be mainly due to the small sample size. The respective decrement in ALT and AST levels was significant only in the control group, perhaps because of an imbalance in the male to female distribution between groups. The NAC group comprised mainly male patients over against female in the control group. Females may have a greater awareness of weight control and cessation of alcohol than males. Also males may under-report their alcohol drinking. There have been two experimental studies which used NAC for the treatment of NASH. The first uncontrolled study showed significant improvements in aminotransferase levels in 11 patients after a 3-month treatment of 1 g NAC/day[12]. The second study was a randomized controlled study conducted in 30 NASH patients[13]. NAC 600 mg/day was administered orally for 4 weeks to15 patients, while the control group (n = 15) was followed up without therapy during that period. In this second study, it was not possible to attribute improvements in the biochemical parameters as therapeutic effects of the drug, possibly because of numerous confounding parameters during the treatment and naturally occurring fluctuations in the ALT and AST levels over the course of the disease. These reasons might explain the results of our study. However, since NAC is a safe and inexpensive orally administered drug, further study with a larger sample size, higher dosage regimen or stratification of changes of the other metabolic parameters included in analytical processes is warranted.

In conclusion, NAC is a safe medication and can be tolerated well in patients with NAFLD, however, our study could not detect any statistically significant change in the level of serum aminotransferase.

REFERENCES

1. Ludwig J, Viggiano TR, McGill DB, *et al*. Nonalcoholic steatohepatitis: Mayo Clinic experiences with hitherto unnamed disease. Mayo Clin Proc 1980;55:434-8.

2. Powell EE, Cooksley WG, Hanson R, *et al*. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 1990;11:74-80.

3. Diehl AM, Goodman Z, Ishak KG. Alcohollike liver disease in nonalcoholics. A clinical and histologic comparison with alcohol induced liver injury. Gastroenterology 1988;95:1056-62.

4. Bedogni G, Bellentani S. Fatty liver: how frequent is it and why? Ann Hepatol 2004;3:63-5.

5. Neuschwander-Terri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37:1202-19.

6. Falck-Ytter Y, Younossi ZM, Marchesini G, *et al*. Clinical features and natural history of nonalcoholic steatosis syndrome. Semin Liver Dis 2001;21:17-26.

7. Kladchareon N, Treeprasertsuk S, Mahachai V, *et al*. The prevalence of nonalcoholic steatohepatitis in Thai patients with non-HBV, non-HCV chronic hepatitis. J Med Assoc Thai 2004;87 (Suppl 2):S29-34.

8. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-5.

9. Reid AE. Nonalcoholic fatty liver disease. In: Sleisenger MH, Fordtran JS, editors. Gastrointestinal and liver disease. 8th ed. Philadelphia: WB Saunders; 2006. p. 1793-1802.

10. Meister A. Glutathione metabolism and its selective modification. J Biol Chem 1988;263:17205-8.

11. Thong-Ngam D, Samuhasaneeto S, Kulaputana O, *et al*. N-acetylcysteine attenuates oxidative stress and liver pathology in rats with nonalcoholic steatohepatitis. World J Gastroenterol 2007;13:5127-32.

12. Gulbahar O, Karasu ZA, Ersoz G, *et al*. Treatment nonalcoholic steatohepatitis with N-acetylcysteine (Abstract). Gastroenterology 2000;118:A1444.

13. Pamuk GE, Sonsuz A. N-acetylcysteine in the treatment of nonalcoholic steatohepatitis. J Gastroenterol Hepatol 2003;18:1220-1.

14. National Cholesterol Education Program Expert Panel. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III): final report. JAMA 2001;285:2486-97.

15. Tan CE, Ma S, Wai D, *et al*. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? Diabetes Care 2004;27:1182-6.

16. American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705-25.

17. Buranawati W, Thoun-U-Thaisri P, Pramoolsinsup C, *et al*. Pentoxyphylline for treatment of nonalcoholic fatty liver disease (NAFLD): a randomized placebo-controlled study. Thai J Gastroenterology 2007;8:57-64.