

The Degree of Insulin Resistance in Hepatitis C Infection

Jaruwat Yossombat, M.D.*

Nattapong Kosachunhanan, M.D.**

Lakkana Thaikruea, M.D.[†]

Wittanee Na Chiang Mai, M.D.^{††}

Satawat Thongsawat, M.D.*

ABSTRACT

Background: The prevalence of type 2 diabetes mellitus is higher in chronic hepatitis C infection with significant higher degree of insulin resistance. There are limited data of insulin resistance in Thai patients with chronic hepatitis C.

Aim: To compare the degree of insulin resistance in non-diabetic chronic hepatitis C patients with matched healthy controls.

Patients and Methods: A case-control study was conducted, comprising 85 confirmed non-diabetic chronic hepatitis C patients who attended for blood donation at Chiang Mai University Hospital and 75 healthy blood donors matched by age and gender. The demographic and the clinical data were collected. Biochemical tests were carried out. Hepatic ultrasonography was performed in all chronic HCV cases, and was combined with clinical and biochemical tests for the exclusion of liver cirrhosis. The degrees of insulin resistance as determined by the homeostasis model assessment (HOMA-IR) were compared between the two groups.

Results: Non-diabetic chronic HCV subjects had no significantly different degree of insulin resistance when compared with matched healthy controls (1.638 ± 1.359 vs 1.612 ± 1.131 , $p = 0.894$). Using the BMI cut-off point of 23 for determining an overweight status for Asian populations, there were no differences of the HOMA-IR in BMI >23 between the two groups (2.27 ± 1.50 vs 2.13 ± 1.83 , $p = 0.666$). There were significantly lower HOMA-IR in HCV cases in BMI <23 compared with healthy subjects (0.86 ± 0.51 vs 1.13 ± 0.56 , $p = 0.027$).

Conclusions: A significantly higher degree of insulin resistance in patients with hepatitis C may not be the case in patients with a younger age of onset or a shorter duration of HCV infection. A lower BMI may be another possible explanation for the lower degree of insulin resistance in this type of patients.

Key words : degree, insulin resistance, hepatitis C

[Thai J Gastroenterol 2005; 6(3): 137-143]

*Division of Gastroenterology, **Division of Endocrinology and Metabolism, Department of Internal Medicine

[†]Department of Community Medicine, ^{††}Department of Radiology

Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

BACKGROUND

There is a growing evidence that chronic infection with hepatitis C virus (HCV) is associated with an increased risk for the development of type-2 diabetes mellitus (DM).⁽¹⁻⁵⁾ Some previous studies indicated that the prevalence of type-2 DM was higher in patients with chronic HCV infection^(3,4). HCV infection was found by multivariate analysis^(6,7) to be an independent risk factor in association with type-2 DM. The mechanism of this association remains unclear. Hyperinsulinemia and insulin resistance play an important role in the development of type-2 DM. These are evident even before the development of impaired glucose tolerance⁽⁸⁾. Therefore, the detection of hyperinsulinemia and of insulin resistance is a good predictor of type-2 DM. Other earlier studies demonstrated increased insulinemia levels in patients with chronic HCV infection compared to controls⁽⁹⁻¹¹⁾. Serrano *et al.*⁽¹²⁾ determined baseline insulinemia in non-diabetic cirrhotic patients infected with HCV, comparing their values with those of a group of non-HCV non-diabetic cirrhotic patients. They concluded that HCV-positive non-diabetic cirrhotic patients had higher baseline insulinemia levels and an increased prevalence of hyperinsulinemia than patients with liver cirrhosis from other etiologies.

Euglycemic-hyperinsulinemic clamp is the gold standard technique for estimating insulin resistance⁽¹³⁾. It is accurate but rather complicated, laborious and in practical. The homeostasis model assessment for insulin resistance (HOMA-IR) has been suggested as another method to assess insulin resistance⁽²¹⁾. It is simply calculated, using just a fasting blood sample drawn for glucose and insulin. It has been well established that HOMA-IR is a useful surrogate index of insulin resistance both in the diabetics and in non-diabetic subjects⁽¹⁵⁻¹⁸⁾. Thus it can be reliably employed in epidemiological studies.

Recently Hui JM, *et al.* demonstrated hyperinsulinemia along with higher HOMA-IR levels in early stages of HCV infection. They also found that HCV genotype-3 described significantly lower HOMA-IR levels than other genotypes⁽¹⁹⁾. A study of hepatitis C infection in Thais showed that the most prevalent genotype was genotype-3 followed by genotype 1 and genotype 6⁽²⁰⁾. As there are distinct geographic variations in the frequencies of different genotypes, and considering ethnic differences in Asian populations as op-

The Degree of Insulin Resistance in Hepatitis C Infection

posed to western populations, we therefore conducted this study to compare the degree of insulin resistance in non-diabetic non-cirrhotic chronic HCV cases with matched healthy controls. We also attempt to identify the possible associated factors, such as the duration of HCV infection, the age of onset, and genotypes as well.

PATIENTS AND METHODS

We performed a case-control study comparing non-diabetic chronic hepatitis C patients with matched normal controls, to evaluate the HOMA-IR between the two groups. Further analysis was also made to look for specific characteristics that may reveal the difference of HOMA-IR.

Case Selection

One-hundred-and-nine blood donors at Chiang Mai University Hospital were recruited between January 2001 and December 2004. The subjects were HCV-positive on screening with HCV enzyme immunoassay (EIA)-3 (Abbott Laboratories, North Chicago, IL). Many subjects also participated in other studies⁽²²⁾. All had a positive HCV-RNA by PCR method (QIAGEN, Gmb H, Hilden Germany). None were diabetic (fasting plasma glucose <126 mg%) and showed no evidence of liver cirrhosis by physical examination (spider nevi, gynecomastia, palmar erythema, testicular atrophy, parotid gland enlargement, Dupuytren's contracture, abnormal biochemical tests and ultrasonography of the liver. No one had a previous treatment of HCV infection. Exclusion criteria included HBV infection, HIV infection, other known liver diseases and the body mass index (BMI) of more than 30 kg/m². The latter was to be excluded due to its nature as it is an independent risk factor for DM.

Clinical and Laboratory Assessment in HCV Cases

All participants were requested to give a written informed consent. The following data were collected at the initial of interview: age, sex, weight, height, and waist-hip ratio (WHR, calculated as waist circumference at umbilicus/hip circumference over the buttocks). The body mass index (BMI) was calculated as weight in kilograms/height in square meters. The average daily alcohol consumption over the past 6 months, familial history of diabetes, the route of HCV transmission, the age of onset and the duration of HCV infection were

also recorded. Intravenous drug usage (IVDU), blood transfusion, tattooing and sexual relation with HCV positive persons were defined as major routes of HCV transmission. Undetermined date of infection or other routes were defined as unknown. The estimated duration of infection was defined as the time elapsed from the presumed date of infection to the date of interview. The onset of infection was determined by the presumed date of the first route of transmission, if the subject appeared to have more than one route of transmission.

All participants were given a full physical examination, focusing on signs of chronic liver disease (spider nevi, gynecomastia, palmar erythema, testicular atrophy, parotid gland enlargement, Dupuytren's contracture), and portal hypertension (ascites, splenomegaly, superficial vein dilatation). The physical examination was conducted by a physician (JY).

All HCV cases had upper abdominal ultrasonography by a radiologist (WN) on the same day or within one week after the interview.

After an overnight fast of 12 hours, venous blood was drawn for the serum levels of liver function tests (albumin, globulin, total bilirubin (TB), direct bilirubin (DB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)), fasting plasma glucose (FPG) and fasting insulin (FI). Serum insulin was determined by (method). The biochemical tests were determined by automated procedures in the laboratories of Chiang Mai University Hospital. The degree of insulin resistance was determined by the homeostasis model assessment (HOMA-IR) method, based on the following equation:

<p>Insulin resistance (HOMA-IR)</p> $= \frac{\text{fasting insulin (mg\%)}}{18} \times \frac{\text{fasting glucose (mmol/L)}}{22.5}$
--

Selection of Controls

Eighty-six anti-HCV negative blood donors from June to December 2004 were enrolled. They were apparently healthy and had no known history of diabetes mellitus. All signed the informed consent. The data collected at the interview, the physical examination, and the biochemical blood tests were performed in the same manners.

The inclusion criteria were :

1. age >18 years,
2. alcohol consumption <30 grams/day,
3. no regular or recent hepatotoxic medication in-

gestion in the previous 2 weeks,

4. non-diabetes (FPG <126 mg%),
5. normal or mildly abnormal LFT (albumin >3 mg%, AST, ALT, or ALP <2 x, TB <2 mg %).

The exclusion criteria were :

1. BMI >30 kg/m²,
2. Presence of any known underlying diseases related to the insulin resistance (hypertension, coronary artery disease, dyslipidemia).

Eighty-six healthy controls were matched by age and gender with the 106 HCV patients. The following data, including BMI, WHR, AST, ALT, fasting plasma glucose, fasting insulin, HOMA-IR were compared between the two groups.

We also compared HOMA-IR between the two groups, looking at age ≥30 vs. <30, male vs. female and BMI ≥23 kg/m² vs. <23 kg/m².

The study was reviewed and approved by the Research Ethics Committee of Chiang Mai University.

Statistical Analysis

Quantitative data were expressed as mean ± SD or as median. Categorical variable data were expressed as frequency and percentage. Comparison of HOMA-IR between the HCV group and the healthy controls were performed, using student's t-test analysis or non-parametric test as appropriate. The Chi-Square test was used to quantify the association between the nominal categorical variables. Independent variables that were considered include age, gender, BMI, WHR, LFT, familial history of DM, age at onset of the HCV infection, duration of HCV infection, amount of alcohol consumption amount and HCV genotypes. P value <0.05 was considered to be statistically significant. All data were analyzed by Epi Info version 6 and SPSS for window.

RESULTS

Of the 109 HCV patients enrolled, 24 cases were excluded [BMI >30 kg/m² (n = 6), fatty liver (n = 9), cirrhosis (n = 4), FPG ≥126 mg% (n = 2), HBV co-infection (n=3)]. Of the 86 controls matched by age and gender enrolled, 8 cases were excluded [BMI >30 kg/m² (n = 9), FPG ≥126 mg% (n = 2)]. Thus, these were 85 confirmed cases and 75 confirmed matched controls (Figure 1). The demographic characteristics of the patients and controls are listed in Table 1.

There were no significant differences of age,

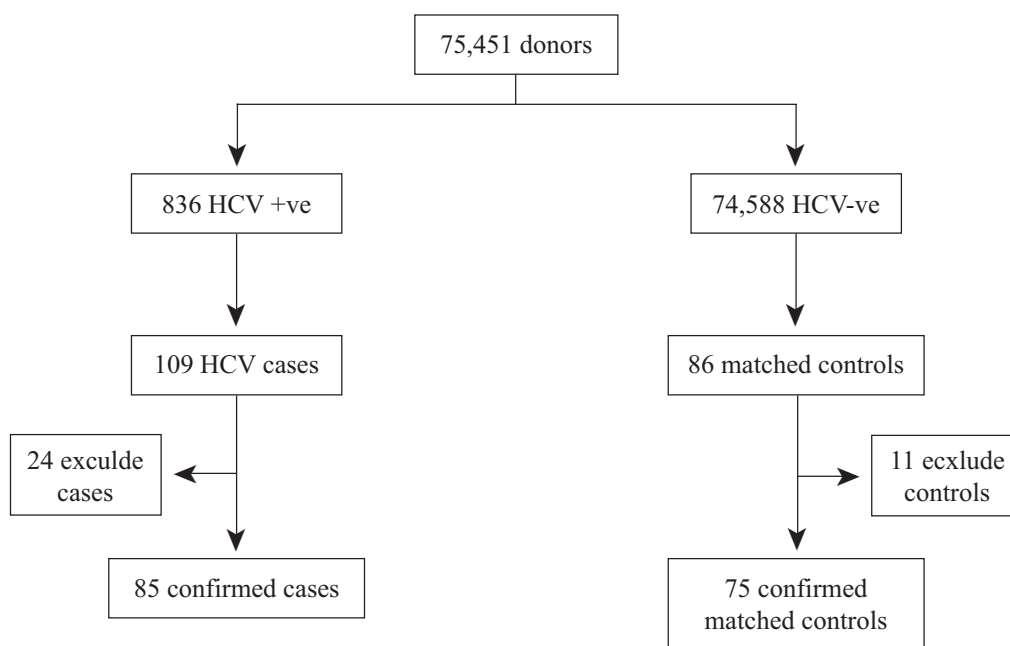


Figure 1 Flowchart of patients recruitment.

Table 1 Demographic data and clinical characteristics of the HCV patients compared with controls.

	Data cases (n = 85)	Controls (n = 75)	p value
Age (years)	37.4 ± 9.9 (19-57)	36.2 ± 9.9 (18-54)	0.421
Sex (male, %)	74 (87.1%)	63 (84%)	0.746
Body weight (kg)	65.2 ± 10.2 (50-95)	64.9 ± 9.8 (47-94)	0.836
BMI (kg/m ²)	23.5 ± 3.0 (17.3-30)	23.2 ± 2.8 (18.6-30)	0.496
WHR	0.87 ± 0.06 (0.75-0.98)	0.86 ± 0.07 (0.62-1.01)	0.663
AST (U/L)*	50 (17-275)	23 (14-85)	0.000
ALT (U/L)*	66 (11-505)	23 (4-88)	0.000
Family history of DM	20 (23.5%)	NA	
Daily alcohol intake in the past 6 months (n = 71)		NA	
>30 gram/day	18 (25.4%)		
<30 gram/day	53 (74.6%)		
Age at onset of HCV infection (n = 55) (years)	22.3 ± 8.5 (8-45)		
Estimate duration of HCV infection (n = 55) (months)*	156 (12-360)		
Route of HCV transmission			
IVDU	25 (29.4%)		
Blood transfusion	25 (29.4%)		
Tattoo	28 (32.9%)		
Sexual contact with HCV person	19 (22.4%)		
Unknown	10 (11.8%)		
1 route	35 (41.2%)		
More than 1 routes	20 (58.8%)		
Genotype	NA		
1			
3			
6			

Note: Data are mean ± SD (range), *median or frequency (%).

Table 2 Comparison of data between HCV patients and matched controls.

Data	Cases (n = 85)	Controls (n = 75)	p value
FPG (mg%)	77.3 ± 7.7 (62-105)	78.7 ± 9.3 (58-114)	0.309
Fasting insulin (uU/mL)*	6.41 (0.94-43.02)	7.09 (1.27-28.92)	0.716
HOMA-IR	1.64 ± 1.36 (0.17-8.29)	1.61 ± 1.13 (0.23-6.78)	0.894

Note: Data are mean + SD (range), *median.

Table 3 Comparison of HOMA-IR between HCV patients and matched controls with different BMI.

Data	HOMA-IR cases	HOMA-IR controls	p value
BMI >23	2.27 ± 1.50 (0.52-8.29)	2.13 ± 1.83 (0.59-6.78)	0.666
BMI <23	0.86 ± 0.51 (0.17-2.02)	1.13 ± 0.56 (0.23-0.81)	0.027

Note: Data are mean + SD (range).

Table 4 Differences of BMI between 2 groups (n = 160).

Data	Cases (n = 85)	Controls (n = 75)	p value
BMI >23	55.3%	48.0%	0.357
BMI <23	44.7%	52.0%	

Note: Data are frequency (%).

gender, body weight, BMI and WHR in groups (p all >0.05). The HCV patients had significant higher levels of both AST and ALT (50 vs. 23, $p = 0.000$ and 66 vs. 23, $p = 0.000$ respectively). There were 23.5% of HCV cases with family history of diabetes mellitus. Daily alcohol intake in the past 6 months ≥ 30 grams/day (level of increasing risk for liver injury) were 25.4% in HCV cases. Reliable data on the date of infection were available in 55 patients. The mean age at onset of HCV infection was 22.3 ± 8.5 years. The mean duration of infection was 170.3 ± 95.7 months. Tattooing was the most common presumed route of HCV transmission (32.9%). About 58.8% of HCV cases were infected by more than one transmission route. The HCV genotype was available in (number) patients.

For an assessment of insulin resistance in hepatitis C virus, there were no differences of the HOMA-IR between the two groups (1.64 ± 1.36 vs 1.61 ± 1.13 , $p = 0.894$) (Table 2). When using BMI cut-off point of 23 kg/m² for determining an overweight status in Asian populations⁽²³⁾, there were no differences of HOMA-IR in BMI ≥ 23 kg/m² (2.27 ± 1.50 vs. 2.13 ± 1.83 , $p = 0.666$). There were significantly lower HOMA-IR in HCV cases in BMI <23 (0.86 ± 0.51 vs

1.13 ± 0.56 , $p = 0.027$) (Table 3). There were also no differences between BMI ≥ 23 kg/m² and <23 kg/m² in both groups (Table 4).

DISCUSSION

Type 2 DM is common in chronic hepatitis C infection. Epidemiological studies showed the increased prevalence of type II DM among chronic hepatitis C patients, and even suggested that HCV infection is an independent risk for developing type II DM⁽¹⁻⁵⁾. As yet, no one was able to explain the mechanism of this process. Some authors tried to demonstrate that chronic hepatitis C patients had a higher prevalence of hyperinsulinemia, and this later on, led to insulin resistance. It is widely accepted that hyperinsulinemia and insulin resistance are found in the early stages of DM, and they may play important roles in the development of type 2 DM⁽⁸⁾.

Some earlier studies found hyperinsulinemia and reduced insulin sensitivity in HCV patients with moderate to severe hepatic fibrosis^(9-12, 24). Recently, Hui, *et al.*⁽¹⁹⁾ extended these findings to HCV patients in the early stage without significant fibrosis from liver biopsy (minimal or no fibrosis). They also found that different genotypes had different HOMA-IR levels.

This study was conducted in Thai chronic hepatitis C patients, who were non-diabetic and were shown not to have developed liver cirrhosis as yet by clinical, laboratory and ultrasonographic criteria. Well matched controls were chosen for comparison. It is interesting that we found no significant differences in the degree

of insulin resistance between the non-diabetic non-cirrhotic Thai HCV patients and the healthy controls.

How may we explain this contradictory findings? Upon reviewing the previous study by Hui *et al.*, we found that their patients were nearly of the same age (37.9 ± 8.9 years) as with our study (37.4 ± 9.9 years). So, age may not be the relevant factor. The other demographic characteristic is obesity (BMI of more than 25 kg/m^2 for Asian populations). It should be noted that in our study those subjects with BMI more than 30 kg/m^2 were not included, and about half of our patients and controls had normal weights (BMI less than 23 kg/m^2), compared with a mean of 26.5 ± 4.7 and a range of $17.2\text{--}47.3 \text{ kg/m}^2$ in the former study. So in this rather not-so-overweight patient group of ours, the risk from hepatitis C chronic infection may not be manifest yet.

One striking difference was that the mean FPG in Hui's study was $93.6 \pm 12.6 \text{ mg\%}$, compared with $77.3 \pm 7.7 \text{ mg\%}$ in ours. Their mean serum insulin level (10.3 uU/mL) was also higher than in our study (median of 6.41 uU/mL). These two values made the HOMA-IR, a product of both values, higher in their study.

Regarding the characteristics of hepatitis C infection, our patients contracted infection at about the same age (22.3 ± 8.5 years) as in Hui's study (22 ± 7 years). Again the age at onset may not be associated with insulin resistance. However, the duration of infection may be important. It is surprising that the median duration of infection in our patients was only 13 years, with a maximal duration of 30 years. While previous reports showed the durations of infection of up to 19 ± 8 years. This suggests that a longer duration of infection may be associated with the development of insulin resistance, and finally of DM.

In this preliminary report we have not obtained data on genotypes yet. (Hui *et al.* noted that genotype 3 had a lower prevalence of insulin resistance. About two-thirds of our patients were of genotypes 3 and 6. Genotype 6 may also have the same characteristic as genotype 3, thus making our patients no different from controls.)

In addition, there may also be some other factors at work, such as different ethnic backgrounds. Data from previous studies were with western populations that may be quite different from Asian populations. Our data also showed a male predominance (87.1%), a factor that may affect insulin resistance.

The Degree of Insulin Resistance in Hepatitis C Infection

One limitation in this study is that we did not obtain liver biopsy. So the data on fibrotic stage was not available. Maeno T, *et al.* demonstrated that HOMA-IR levels increased in parallel with the progression of fibrosis⁽²⁵⁾. It was possible that we included a fairly large number of patients with early stages of fibrosis, hence the lower HOMA-IR levels than in previous studies. However, this is not certain. Further study is needed to clarify this association.

Since our study did not show the difference in insulin resistance between patients with chronic hepatitis C infection and normal controls, it would be useful if further study would be conducted in larger patient populations, including larger numbers of overweight patients, and appropriate numbers of different HCV genotypes for a more meaningful analysis.

REFERENCES

1. Mason AL, Lau JY, Hoang N, *et al.* Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; 29: 328-33.
2. Allison ME, Wreghitt T, Palmer CR, *et al.* Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1987; 21: 1135-9.
3. Grimbirt S, Valensi P, Levy-Marchal, *et al.* High prevalence of diabetes mellitus in patients with chronic hepatitis C: A case-control study. *Gastroenterol Clin Biol* 1996; 20: 544-8.
4. Ozyilkan E, Erbas T, Simsek H, *et al.* Increased prevalence of chronic hepatitis C virus antibodies in patients with diabetes mellitus. *J Intern Med* 1994; 235: 283-4.
5. Alexander GJ. An association between hepatitis C virus infection and type 2 diabetes mellitus: what is connection? *Ann Intern Med* 2000; 133: 650-2.
6. Knobler H, Schihmanter R, Zifroni A, *et al.* Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. *Mayo Clin Proc* 2000; 75: 355-9.
7. Caronia S, Taylor K, Pagliaro L, *et al.* Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; 30: 1059-63.
8. Martin BC, Warram JH, Krolewski AS, *et al.* Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 1992; 340: 925-9.
9. Garrido Serrano A, Guerro Igea FJ, Lepe Jimenez JA, *et al.* Hyperinsulinemia in cirrhotic patients infected with hepatitis C virus. *Gastroenterol Hepatol* 2001; 24: 127-31.
10. Furutani M, Nakashima T, Sumida Y, *et al.* Insulin resistance/ beta-cell function and serum ferritin level in non-diabetic patients with hepatitis C virus infection. *Liver Int* 2003; 23: 294-9.

11. Sougleri M, Labropoulou-Karatza C, Paraskevopoulou P, *et al.* Chronic hepatitis C infection without cirrhosis induces insulin resistance in patients with alpha-thalassaemia major. *Eur J Gastroenterol Hepatol* 2001; 13: 1195-9.
12. Garrido Serrano A, Guerrero Igea FJ, Lepe Jimenez JA, *et al.* Hepatitis C virus infection, increased serum ferritin and hyperinsulinemia. *Rev Esp Enferm Dig* 2001; 93: 639-48.
13. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. *Endocr Rev* 1985; 6: 45.
14. Elahi D. In praise of the hyperglycemic clamp. A method for assessment of beta-cell sensitivity and insulin resistance. *Diabetes Care* 1996; 19: 278-86.
15. Yokoyama H, Emoto M, Fujiwara S, *et al.* Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment in normal range weight and moderately obese type 2 diabetic patients. *Diabetes Care* 2003.
16. Bonora E, Targher G, Alberiche M, *et al.* Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000; 23: 57-63.
17. Ikeda Y, Suehiro T, Nakamura T, *et al.* Clinical significance of the insulin resistance of the insulin resistance index as assessed by homeostasis model assessment. *Endocr J* 2001; 48: 81-6.
18. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 1997; 20: 1087-92.
19. Hui JM, Sud A, Farrell GC, *et al.* Insulin resistance is associated with chronic hepatitis C and virus infection fibrosis progression. *Gastroenterology* 2003; 125: 1695-704.
20. Verachai V, Phutiprawan T, Theamboonlers A, *et al.* Prevalence and genotypes of hepatitis C virus infection among drug addicts and blood donors in Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 849-51.
21. Matthews DR, Hosker JP, Rudenski AS, *et al.* Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412-9.
22. Thaikruea L, Thongsawat S, Maneekarn N, *et al.* Risk factors for hepatitis C virus infection among blood donors in Northern Thailand. *Transfusion* 2004; 44: 1433-40.
23. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies WHO expert consultation. *Lancet* 2004; 363: 157-63.
24. Petit JM, Bour JB, Galland-Jos C, *et al.* Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* 2001; 35: 279-83.
25. Maeno T, Okumura A, Ishikawa T, *et al.* Mechanisms of increased insulin resistance in non-cirrhotic patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2003; 18: 1358-63.