Research Article

Mutual Relationship between Some Metabolic Factors and Chronic Hepatitis C Infection in Relation to the New Directly Acting Antiviral Drugs.

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Abstract
Background: Chronic HCV infection is associated with altered metabolism, including dyslipidemia and insulin resistance, which contributes to disease progression and influences response to therapy. **Aim:** To investigate the relationship of new direct acting antiviral drugs (DAAs) with lipid profile and insulin resistance. **Methods:** Eighty chronic hepatitis C (CHC) genotype 4 patients were included; they were divided into 4 groups according to the severity of fibrosis as detected by fibroscan. Forty healthy volunteers were selected as a control group. Changes in the levels of lipid profile and insulin resistance during treatment with simeprevir/ sofosbuvir (SIM/SOF) were analyzed and effect on response was studied. **Results:** An end of treatment response was achieved in (99%) of the treated cases with 99% SVR-12. The levels of serum triglycerides were significantly higher in patients compared to control. The levels of fasting insulin increased progressively with increasing stage of fibrosis. At end of treatment, There were significant reduction in serum triglycerides, FBS, fasting insulin, HOMA- IR ($P<0.001$), and a significant elevation of serum cholesterol and LDLC, HDL c levels ($P= 0.001$). **Conclusion:** Serum lipid levels and insulin resistance are no longer predictors of response to DAAs. There were significant changes in lipid profile at 12 weeks after treatment. Lipid profile should be followed after treatment with DAAs.
Keywords: Chronic Hepatitis C, Direct acting antiviral drugs, Lipid profile, Insulin resistance

Introduction
HCV is the major causative agent of chronic liver diseases. More than 170 million patients worldwide were estimated to suffer chronic liver disease after HCV infection[1]. CHC can be considered a special type of metabolic disease involving insulin resistance (IR), hepatic steatosis and modulation of lipid-cholesterol biosynthesis as fatty liver, hypo-betalipopro-teinemia, hypercholesterolemia[2], and increased risk for ischemic heart diseases was reported[3]. The association between chronic HCV and increased prevalence of IR and type 2 diabetes mellitus (DM) was extensively reported[4]. IR was reported to accelerate fibrosis in chronic HCV patients[5,6]. It increases the risk of cirrhosis and hepato-cellular carcinoma[7] and has been associated with a reduced rate of sustained virological response (SVR) in response to pegylated interferon (IFN)-α and ribavirin therapy[8].

In most countries, treatment of chronic HCV infection is shifted from IFN-based to IFN-free regimens composed of DAAs. Although the association of baseline meta-bolic characteristics with treatment outcome has not been fully assessed for DAAs, they were reported to result in improved rates of SVR and reduce the predictive ability of these factors except for the baseline LDL[9]. The highest prevalence of HCV was reported in Egypt, where genotype 4 is responsible for 91% of infections[10] and the treatment is composed of DAAs in most of its centers[11]. Although some studied lipid metabolism changes after DAAs treatment[12,13], no one up to our knowledge studied these changes in genotype 4 infected patients. The aim of this study was

to evaluate the outcome of using SIM/SOF in treating these patients and to study the relationship between new DAAs (SIM/SOF) and lipid profile and insulin resistance.

Patients and methods

Eligible patients

The study retrospectively assessed 80 treatment-naïve patients with compensated CHC who attended the liver unit of Minia University Hospital, Minia, Egypt with approval from the national committee for control of viral hepatitis.

The target population included patients with chronic hepatitis C, with different fibrosis grades (F), treated with (SIM/SOF). Grades of fibrosis were estimated using FibroScan.

Treatment protocol:

Treatment of patients was achieved with SIM capsules 150mg once a day and SOF tablets 400 mg once a day, and it was continued for 12 weeks. The SOF dose was changed to 200 mg once a day if estimated glomerular filtration rate dropped below 30 mL/min at the discretion of the treating physician. The study was conducted during the period from February 2015 to January 2016.

Criteria of subjects:

Eligible patients were ≥18 years of age that had CHC based on the presence of anti-HCV and detectable serum HCV-RNA for 6 months or more. All patients were infected with HCV-4. Exclusion criteria included patients with diabetes mellitus and patients with other causes of liver disease including: concomitant hepatitis B virus, human immunodeficiency virus, schistosoma co-infection, autoimmune hepatitis and patients with alcohol intake >40 g per day in the last 6 months prior to the start of antiviral treatment.

Informed consent

The study protocol was approved by the Institutional Ethics Committee of participating center. Informed consent was obtained from all individual participants included in the study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Assessment of HCV-RNA load and viral kinetics

HCV RNA level determination was done at baseline, at end of treatment (week 12) and 12 weeks after completion of treatment. HCV RNA was measured using the Roche COBAS TaqMan HCVassay V.2.0 (lower limit of detection 15 IU/mL). Virological outcomes included achievement of undetectable HCV RNA at end-of-treatment response (EOTR), defined as undetectable HCV RNA at the completion HCV therapy. SVR12 was defined as an undetectable HCV RNA 12 weeks after completion of HCV therapy. HCVRNA levels were quantified with a lower limit of detection of 15 IU/mL.

Clinical and laboratory assessment:

History and clinical examination were taken with special stress on age, gender, daily alcohol intake in the past 6 months (g/day), route of HCV transmission, body mass index (BMI), and waist circumference. BMI was calculated as weight divided by the square of the height (kg/m^2). The waist circumference was measured 1 inch above the navel or midpoint between the lower margin of the least palpable rib and the top of the iliac crest parallel to the floor, while the hip circumference was measured at its widest part of the buttocks or hip parallel to the floor. Venous blood was drawn after at 8 hours overnight fast to determine the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, bilirubin, platelet count, international normalized ratio (INR), Serum glucose and insulin then patients completed fasting for 12 hour for assessment of total cholesterol (TC), HDL, LDL cholesterol, triglycerides. Total and HDL cholesterol and triglycerides were determined by automated procedures. Serum insulin was determined by electro-chemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, Indiana, USA). Insulin resistance was then investigated in all patients by the homeostasis model for the assessment of IR (HOMAIR) using the

standard formula: HOMA-IR = fasting insulin (uU/mL) x fasting glucose (mmol/L)/22.5. HCV genotype was detected using a line probe assay or reverse hybridization (InnoLipa; Innogenetics, Genetics, Gent, Belgium).

Statistical analyses

Symmetrically distributed continuous variables were summarized as a mean ± standard deviation (SD). The median and interquartile ranges were used for skewed continuous variables. Categorical variables were presented as frequency and percentage. Comparisons between groups were made by using the Mann–Whitney U test or the Student *t* test for continuous variables and the χ^2 or Fisher exact probability test for categorical data. The two-tailed paired Student's *t*-test was used to test for significance of differences between baseline and post-treatment lipid profile and metabolic factors, HOMA-IR. The Pearson correlation coefficients were used to study the correlation between different parametric variables. Spearman rank correlation was used to quantify the association between continuous or ordered categorical variables. Logistic regression analysis was used to model the association between baseline lipid profile, HOMA-IR and other covariates to determine the factors associated with hepatic fibrosis, also for determination of the factors associated with changes in LDL-c. $P \leq 0.05$ was considered statistically significant. SPSS software for Windows version 20 (SPSS Inc., Chicago, IL) was used to perform all analyses.

Results

Demographic and base line characteristics:

We studied 80 Egyptian patients with chronic hepatitis C (genotype 4), who were treated with SIM+SOF. Their demographic, biochemical, metabolic and fibroscan data were detailed in (Table 1). The mean age was 47.5±12.3 years (range 20- 67 years). Of them, 48 patients were males and 32 were females. The mean BMI was 22.2±1.8 kg/m² (range 16-26 kg/m²) and the mean waist/Hip Ratio was 0.93±0.019 (range 0.9-0.97). HOMA- IR > 3 was documented in 45% of patients. Based on fibroscan examination, 34 patients had mild fibrosis (15 cases were F1 and 21 cases were F2), while 44 patients had moderate to severe fibrosis (16 cases were F3 and 28 cases were F4) (Table 1).

Lipid and metabolic factors:

The levels of serum triglycerides, fasting blood glucose, fasting insulin and HOMA-IR were significantly higher in patients compared to control (P value 0.006, 0.01, 0.001 & 0.001 respectively). There is a significant increase in fasting insulin level with increasing stage of hepatic fibrosis. No significant differences were detected in the levels of lipid profile, fasting glucose and HOMA-IR according to stage of fibrosis (Table3).

Changes in laboratory data, Imaging, Metabolic and Lipid Markers:

Patients who achieved ETR showed significant reduction in blood hemoglobin and serum AST and ALT levels (P= 0.001), as well as significant reduction in fibrosis stages, which were clearly shown in FEB4 and fibroscan (P=0.03 and 0.001 respectively) (Table 4). A significant reduction in serum triglycerides, FBS, fasting insulin and HOMA- IR (P<0.001), and a significant elevation of serum cholesterol, LDLC and HDLc levels (P= 0.001) were also noted in treated patients (Table 5).

Factors Associated with ΔLDL-C value in CHC after 12 weeks of the start of therapy of SOF/SIM treatment:

Patients were divided into two groups stratified by their ΔLDL-C levels. The high ΔLDL-C group was defined as having a ΔLDL-C level ≥80.0 mg/dl, using the median value of all subjects. The low ΔLDL-C group was thus defined as having a ΔLDL-C level <78.0 mg/dl. 42 cases have a median >78 and 32 cases have a median <78. We performed a multiple logistic regression analysis to identify the factors associated with ΔLDL-C elevation at 12 weeks from the start of therapy (Table 6). We performed a step-wise logistic regression analysis to determine whether increased LDL-C was associated with age, gender, BMI, AST, ALT, albumin, bilirubin, hemoglobin, platelets, white blood cell count, log viral load, fasting sugar, fasting insulin, HOMA-IR, total

cholesterol, triglycerides and HDL. The results are shown in Table 6. TC and stage of fibrosis, detected by fibroscan, were closely associated with the ΔLDL-C values [OR (95% CI: 1.066 (1.031-1.102) & 1.162 (1.018-1.328)] (p≤0.001 & 0.026) respectively.

Table 1: Demographic and base line characteristics of chronic hepatitis C patients

Variable	Patients (n= 80)
Age (Years)	47±12(20-67)
Gender (Male/Female)	47/33
BMI (KG/m²)	22.28±1.9 (16-25)
Hypertension (Yes/No)	6/59 (9.2%/90.8%)
Smoking (Yes/No)	8/72 (10%/90%)
Waist/Hip Ratio	0.93±0.019 (0.9-0.97)
Hemoglobin (g/dL)	10-17 (13.6±1.3)
Platelets (× 10^9)	194.4±58 (81-430)
Albumin (gm/L)	4.15±0.6 (2.1-5.4)
INR	1.1±0.1(0.9-1.4)
Creatinine (mg/L)	0.94±0.18(0.64- 1.6)
Total Bilirubin (mg/L)	0.46±0.2 (0.1-1.2)
ALT (Iu/L)	50.1±20.0 (21-103)
AST (Iu/L)	50.8±25.8 (17-163)
AFP	3.6±3.8 (0.7-32.8)
Fasting Glucose (mmol/dL)	5.04±0.75 (3.33-6.67)
Insulin Level (uiu/ml)	20.15±5.13(5-29)
HOMA-IR	4.49±1.28 (1.25- 7.25)
HOMA-IR(< 3 & ≥3)	(33&47)(58.8% & 41.3%)
Triglycerides (mg/dL)	98±40.8 (35-225)
HDLC (mg/dL)	42.1±5.8 (31-58)
LDL-C (mg/dL)	63 ± 29 (11- 131)
Cholesterol (mg/dL)	126±28 (70-195)
Viral Load (log10)	5.2+1.3 (2.04-7.9)
Fibroses stage (Fibro scan):	
F1	15 (18.8%)
F2	24 (24%)
F3	17(21.2%)
F4	24 (35%)
(F1, F2&F3, F4)	(39-41) (48.8% -51.2%)
FIB-4	1.9±1.1

BMI: body mass index, ALT: alanine transaminaseAST: aspartate transaminase INR: International normalized ratio, HOMA IR: Homostatsis model assessment of insulin resistance, HDLC: high density lipoprotein cholesterol, AFP: alpha foetoprotein, U/S: ultrasound. P value≤0.05 is significant.

Table 2: Baseline metabolic data, lipid profile in chronic hepatitis C (CHC) patients versus control group

Variable	Patients (n=80)	Controls (n=40)	P-value
Cholesterol(mg/dL)	126±28	137±30.8	0.1
TG (mg/dL)	100.5±36.1	82±27	0.006
LDL-c	63.4±29	79.6±33.7	0.3
HDL-c	39.4±5.3	45.2±5.23	0.776
Fasting Glucose(mmol/dL)	5.04±0.75	4.7±0.52	0.01
Fasting insulin Level (uiu/ml)	20.15±5.13	13.15±4.2	0.001
HOMA-IR	4.49±1.28	2.72±0.87	0.001

TG: triglycerides, LDL- c: low density lipoprotein cholesterol, HDLc: high density lipoprotein cholesterol, HOMA IR: Homostatsis model assessment of insulin resistance, P value≤0.05 is significant.

Table 3: Metabolic factors, lipid profile, according to fibrosis stage in Chronic Hepatitis C patients

Variable	(F0 to F2) n (36)	(F3toF4) n (44)	P-value
Cholesterol (mg/DL)	127±28	125±28	0.9
TG(mg/dL)	102.4 ±39	99.32±4	0.7
LDL-c	63.8±27.5	62±30	0.4
HDL-c	39.3±5	39.6±5.4	0.5
Fasting glucose (mmol/dL)	5.03±0.81	5.06±0.7	0.895
Fasting Insulin (uiu/ml)	19.2±5.4	21.7±4.4	0. 025*
HOMA-IR	4.28±1.3	4.7±1.24	0.154

TG: triglycerides, LDL-c: low density lipoprotein cholesterol,HDLc :high density lipoprotein cholesterol, HOMA IR: Homostatsis model assissement of insulin resistance, P value ≤0.05 is significant.

Table 4: Changes in laboratory data and imaging before and at end of treatment with SOF/SIM (12 months) in CHC patients

	Before Treatment	End of Treatment	P
Hb (g/dL)	13.6±1.3	12.7±1.5	0.001*
Platelets (x 109 /L)	196.09±59.1	194.6±37.06	0.7
ALT(IU/L)	49.8±21.4	41.6±14.4	0.001*
AST(IU/L)	50.2±25.2	40.2±17.3	0.001*
FIB-4	1.9±1.08	1.7±1.1	0.03*
Fibroscan	13.6±10.4	12.6±8.8	0.001*

TG: triglycerides, LDL-c: low density lipoprotein cholesterol, HDLc: high density lipoprotein cholesterol, HOMA IR: Homeostasis model assessment of insulin resistance, P value ≤0.05 is significant.

Table (5): Changes of metabolic factors, lipid profile before and after end of treatment of SOF/SIM treatment (12 weeks) in CHC patients

	Before treatment	End of Treatment	P
TG (mg/dl)	100.5±36	81.7±31.9	0.001*
Cholesterol (mg/dl)	126.1±28.2	143±38.5	0.001*
LDL(mg/dl)	63.4±28.9	84.8±38.1	0.001*
HDL(mg/dl)	39.4±5.3(28-51)	42.9±5.9 (30-58)	0.001*
FBS(mmol/L)	5.04±0.75	4.7±0.52	0.001*
Fasting insulin(ųU/ml)	20.15±5.13	15.7±7.4	0.001*
HOMAIR	4.49±1.28	3.06±0.01	0.001*

TG: triglycerides, LDL-c: low density lipoprotein cholesterol, HDLc: high density lipoprotein cholesterol, HOMA IR: Homeostasis model assessment of insulin resistance, P value ≤0.05 is significant.

Table (6): Factors Associated with ΔLDL-C value with HCV administered SOF/SIM regimen, Analyzed by Multiple Logistic-regression Analysis

	Sig.	OR (95% CI)
Age (Ys)	0.552	1.012 (0.974-1.051)
BMI(Kg/M2)	0.537	0.868(0.554- 1.361)
Gender(male/female)	0.198	1.897(0.716-5.029)
ALT (Iu/L)	0.322	0.974(0.926-1.026)
AST(Iu/L	0.559	0.986(0.939-1.035
TBILIROBIN (mg/L)	0.300	2.972(0.380-23.267)
Albumin(g/L)	0.647	0.734(0.195-2.763)
Hemoglobin (g/dL)	0.987	0.995(0.535-1.849)
Platelets (x 109 /L)	0.677	0.997(0.983-1.011)
WBCs	0.771	1.000 (1.000- 1.000)
Log of viral load(log10)	0.429	1.000 (1.000- 1.000)
HOMA- IR	0.179	0.601(0.286-1.263)
Fasting insulin(uiu/ml)	0.395	0.859(0.606-1.219)
Fasting sugar (mmol/dL)	0.919	1.093(0.196-6.097)
HOMA- IR	0.305	2.948(0.374- 23.229)
Cholesterol(mg/dL)	0.001	1.066(1.031-1.102)
TG(mg/dL)	0.342	0.989(.967- 1.012)
HDLC (mg/dL)	0.435	0.941(.807- 1.097)
Stage of fibrosis (F1-F4)	0.026	1.162(1.018-1.328)

BMI: body mass index, ALT: alanine transaminaseAST: aspartate transaminaseHOMA IR: Homostatsis model assessment of insulin resistance,HDLC: high density lipoprotein cholesterol, P value≤0.05 is significant.

Figure 1: changes in lipid profile in chronic hepatitis C patients before and at end of treatment with SOF/SIM

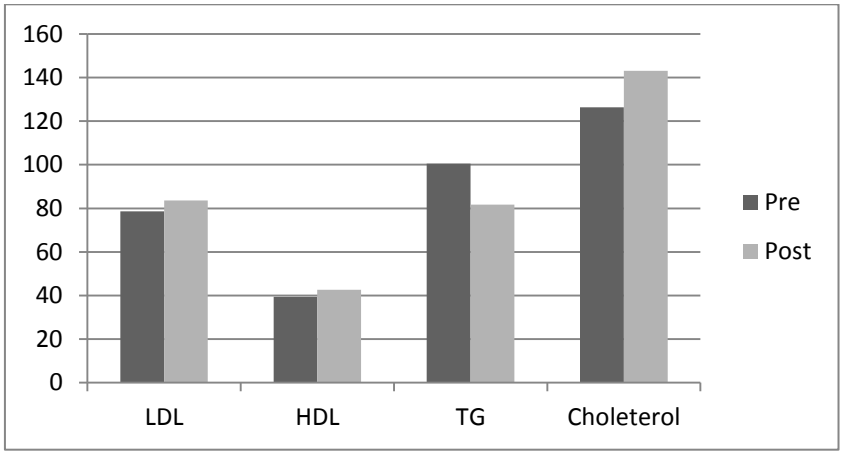

Figure 1 shows changes in lipid profile (mg/DL) in chronic hepatitis C (HCV) patients before and at end of treatment (12 weeks) with SOF/SIM . LDL-c: low density lipoprotein cholesterol, HDL: high density lipoprotein cholesterol, TG: triglycerides

Discussion

DAAs got high satisfaction to be the most accepted treatment scenario for chronic HCV infection that improved the SVR up to 100%. In conjunction with this growing confidence, many of the effects and relationships of these drugs continue to emerge with the continuous research, but many of these effects did not reach the level of the facts and are still controversial. In concordance with Buti et al.,[14] the present study showed an end of treatment response of 99% of the treated cases and 99 % SVR-12 rate. Based on these results, the role of metabolic factors as predictors of the DAAs has been decreased or even ameliorated. The changes in lipid profile after therapy represent an interesting finding which recently reported with the use DAAs[12,15]. We reported a significant reduction in serum triglycerides, and a significant elevation of serum cholesterol, LDL, and HDL levels at 12 weeks after the start of treatment with DAAs.

The well-established direct link between HCV and host lipoproteins explicates the significant interrelationship between HCV and host lipid metabolism; this was proved both in-vitro[16] and clinical studies[12]. In one study, after 24 weeks of treating HCV genotype 1-infected patients with SOF/RBV, concomitant decrease in triglycerides and VLDL particle size and marked increase in serum LDL-C was reported irrespective of treatment outcome[12]. This might explain a direct influence of HCV clearance on lipid metabolism. Upon treating HIV/HCV-co-infected patients with ledipasvir and sofosbuvir combination therapy for 12 weeks, Townsend et al.,[13] reported a rapid increase in both LDL-C and TC values that was sustained after treatment. That increment in serum LDL-C and TC did not differ between HIV/HCV-co-infected and HCV mono-infected patients. Also, Mauss et al.,[15] reported that suppressing and eliminating HCV with IFN-free DAA regimens increased TC levels with no effect on triglycerides. However, with IFN-based therapy, reduction in TC levels and increased triglycerides was found during treatment, followed by increased TC levels after achieving sustained virological response. The changes in lipid profile may be affected by type of DAA used as suggested by Hashimoto et al.,[17]. They studied CHC genotype 1 patients, and observed a rapid increase in LDL-C and TC during the first 28 days of treatment that was stronger in patients received ledipasvir and sofosbuvir than those who received daclatasvir (DCV) and a sunaprevir (ASV).

Endo D et al.,[18] reported significant greater increase in TC and LDL-C in the SOF + LDV group than in the DCV + ASV group during antivirus treatment, while at 4 and 12 week after the therapy, serum levels of TC and LDL-C were similar between the two groups .

Factors that could predict changes in LDL were interred in logistic regression analysis. The main independent predictor for LDL changes in our study was advancing stage of fibrosis and Lower cholesterol levels, This finding comes with the report of Khattab et al.,[19] who studied CHC patients genotype 4 and reported that patients with severe fibrosis had lower HDL-C and cholesterol level than those with mild hepatic fibrosis, a finding which support the possible predictive role of hepatic fibrosis for marked LDL –C change at end of treatment, also the more prominent increase in serum cholesterol and LDL levels in treated patient with advanced fibrosis might represent improvement in liver pathology or inflammation.

HCV infection showed a complex relationship with IR. IR appears at early stages of HCV infection and increases the rate and progression of hepatic fibrosis through compensatory hyper-insulinemia, hepatic stellate cells (HSCs) increment, and type 1 collagen expression proliferation[20]. Also, Hsu et al.,[21] reported a correlation between HCV-RNA levels and HOMA score. Even in non-diabetic patients, Moucari et al.,[22] reported IR in 32.4%, of CHC patients. Despite being non-obese, non-diabetic, or prediabetic 45% of our patients had IR >3. Also, the mean fasting insulin and HOMA-IR were significantly higher in the patients group comparison to control subjects (20.15±5.13 vs 13.15±4.2 p= 0.001) and (4.49±1.28 vs 2.72±0.87 with p=0.001) respectively. Moreover, the fasting insulin showed progressive increase, with increasing stage of hepatic fibrosis which was reported be Petit et al.,[6] who stated that IR in non-diabetic HCV-infected patients was related to grading of liver fibrosis and may occur early in the course of HCV infection.

Although no recent studies addressed the relationship of IR and new DAAs, the high rate of SVR to SOF/SIM-based therapy in our CHC naïve patients means that IR is, no longer, having any predictor role for SVR as that was with the peg-interferon and ribavirin regimens. This concept was supported by earlier studies as reported by Grasso et al.,[9].The metabolic factors seem to have a minor role in influencing antiviral response based on the analysis of the study performed by Serfaty et al.,[23] who used telaprevir-based regimens in patients with genotype 1 CHC. They reported that IR measured by HOMA index did not show any relation to SVR. Also HOMA- IR were not found to be associated with SVR in many other studies [9,24] but they reported significant improvement of IR in HCV genotype 1 infection, in patients who achieved SVR, compared with patients who did not obtain viral clearance after treatment. The same findings were reported by Thompson et al.,[25] and Delgado-Borrego et al.,[26]. Arase et al.,[27] and Aghemo et al.,[28] revealed that achieving viral clearance significantly reduce the risk of both type 2 DM and de novo IR in non-diabetic HCV patients. Similarly our study showed significant improvement of fasting insulin and HOMA-IR in patients who achieved SVR

Our study has many limitations. Of which, the limited number of cases, and we did not conduct an analysis of dynamic changes of LDL and insulin resistance at early time of therapy, Also, we cannot covered a longer term, and whether the changes in LDL-C levels continue after the completion of the treatments used in this population.

Conclusion

The SOF/ SIM based therapy is highly effective treatment for CHC GT4. The high SVR ameliorate predictive effect of the metabolic factors as IR. The rapid and obvious changes in lipid factors is an interesting finding, Long-term follow up for lipid changes is warranted to avoid possible remote effect of CHC as a potential risk for atherosclerotic heart disease.

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