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Research Article

Measures of insulin resistance and beta cell function before and after treatment of HCV infection

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ABSTRACT

The association between chronic HCV infection and type 2 diabetes mellitus (T2DM) has been established; however, there is limited research on β-cell function particularly in the pre-diabetic population. Here, we evaluated indices of β-cell function and insulin sensitivity across the spectrum from normal glucose tolerance to T2DM in individuals with and without chronic hepatitis C (CHC), and the effects of antiviral treatments on these variables. A total of 153 non-cirrhotic, non-fibrotic CHC patients with a BMI <25 were enrolled in the study. Among them, 119 were successfully treated with either direct acting antiviral (DAA) drugs or pegylated interferon/ribavirin (IFN/RBV) anti-HCV therapy. Fasting state- and oral glucose tolerance test (OGTT)-derived indexes were used to evaluate β-cell function and insulin sensitivity. Among all subjects, 19 (13%) had T2DM and 21% exhibited pre-diabetes including 8% isolated impaired fasting glucose (IFG) and 13% combined IFG and impaired glucose tolerance (IGT). Early and total insulin secretion adjusted for the degree of insulin resistance were decreased in pre-diabetic CHC patients compared to HCV-uninfected individuals. Viral eradication through DAA or IFN/RBV therapy demonstrated positive impacts on insulin sensitivity and β-cell function in CHC patients who achieved sustained virologic response (SVR), regardless of fasting or OGTT state. These findings emphasize the role of HCV in the development of β-cell dysfunction, while also suggesting that viral eradication can improve insulin secretion, reverse insulin resistance, and ameliorate glycemic control. These results have important implications for managing pre-diabetic CHC patients and could prevent diabetes-related clinical manifestations and complications.

1. Introduction

According to clinical investigations, diabetes mellitus (DM) and chronic hepatitis C virus (HCV) infection are closely related conditions (Cacoub et al., 2018; Dyal et al., 2016; Mahale et al., 2018; Naing et al., 2012; Shintani et al., 2004). HCV infection is considered as an independent risk factor for the development of diabetes (Mason et al., 1999). It is generally accepted that the induction or exacerbation of insulin resistance by HCV is the mechanism by which it is linked to diabetes (Lecube et al., 2006; Shintani et al., 2004). Impaired insulin secretion has also been found to significantly influence the occurrence of diabetes (Caronia et al., 1999). Intervention studies have shown that maintaining β-cell function lowers the pace at which impaired glucose tolerance (IGT) converts to type 2 diabetes mellitus (T2DM), highlighting the crucial role of β-cell dysfunction in this process. While it is possible that β-cell malfunction may contribute to diabetes in individuals with chronic hepatitis C (CHC), further research is needed to confirm this hypothesis (Narita et al., 2004).

The transition from early metabolic abnormalities including impaired fasting glucose (IFG) and IGT to diabetes may take many years. However, current estimates indicate that most individuals (perhaps as many as 70%) with these pre-diabetic states will eventually develop diabetes (Nathan et al., 2007). In pre-diabetes, the risk of cardiovascular disease

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and long-term complications affecting the eyes, kidneys and nervous system is greatly increased (Schlesinger et al., 2022). Therefore, it is important to include cohorts representing the spectrum from IFG to T2DM to better understand HCV infection-induced β-cell dysfunction during diabetes development.

Effective eradication of HCV improves glycemic control, particularly in individuals achieving sustained virologic response (SVR) with direct acting antiviral (DAA) therapy (Aghemo et al., 2012; Dong et al., 2018). Publications have demonstrated early declines in fasting blood glucose and glycated hemoglobin during and after HCV treatment (Carnovale et al., 2019). Moreover, a prospective case-control study showed an improvement in insulin resistance following the clearance of HCV by DAAs (Brandman et al., 2012). However, the impact of virus elimination on β-cell function remains incompletely elucidated.

In an effort to further evaluate the pathophysiology of the pre-diabetic and diabetic state in patients with CHC, we examined β-cell activity and assessed the variations in insulin secretion across different glucose tolerance subgroups, ranging from normal glucose tolerance (NGT) to T2DM. The measurements were also utilized to compare the impact of HCV eradication on β-cell function, aiming to investigate the interplay between HCV, DM, and anti-HCV therapy. These findings may have significant implications for preventive management strategies and prognostic assessment in CHC patients.

2. Materials and methods

2.1. Patients and the cohort of patients

Nondiabetic HCV-infected subjects aged 18 to 60 with detectable hepatitis C viral load (HCV RNA) were recruited from the First Affiliated Hospital of Xinjiang Medical University's Health Management Center between January 2014 and January 2019. The 205 participants showed no signs of cirrhosis or fibrosis as determined by computed tomography (CT) or ultrasound findings. The diagnosis of chronic HCV infection was made based on standard serological testing and the observation of aberrant serum aminotransferase levels for at least six months. Serum HCV RNA levels were assessed by the quantitative Amplicor HCV test (Roche Molecular System). The patients were divided into three groups according to HCV viral load: low (<100 kcopies/mL), moderate (100–500 kcopies/mL), and high (>500 kcopies/mL). HCV genotype was determined using the reverse hybridization line-probe assay. Patients who met the following requirements were disqualified from the study: HBV surface antigen (HBsAg) positivity, a family history of diabetes (parents and siblings), taking medications that may impair insulin secretion or glucose tolerance, abnormal thyroid tests (thyroid stimulating hormone, free T3, and free T4), abnormal renal function tests (serum creatine levels >1.0 mg/dL), a history of gastrectomy or chronic pancreatitis, a body mass index (BMI) greater than 25, and alcohol consumption more than 50 g per day. Anti-HCV antibodies were another requirement for inclusion (as determined by a third-generation enzyme-linked immunosorbent test). A total of 153 CHC patients were enrolled in the study cohort (Fig. 1). Due to migrant labour, relocation, or other factors, 119 out of 153 CHC patients were included in the HCV therapy research. The treatment plans were based on their particular preferences (Fig. 1). At the time of data collection, the patients had a BMI under 25 and no cirrhosis or fibrosis, as determined by CT or ultrasound. Co-infected patients with HIV and hepatitis B as well as those exhibiting histologic or clinical symptoms of cirrhosis or decompensated liver disease were excluded from the study. A once weekly subcutaneous injection regimen of PEG-IFNα-2a (Pegasys; Roche, 180 μg) was planned for

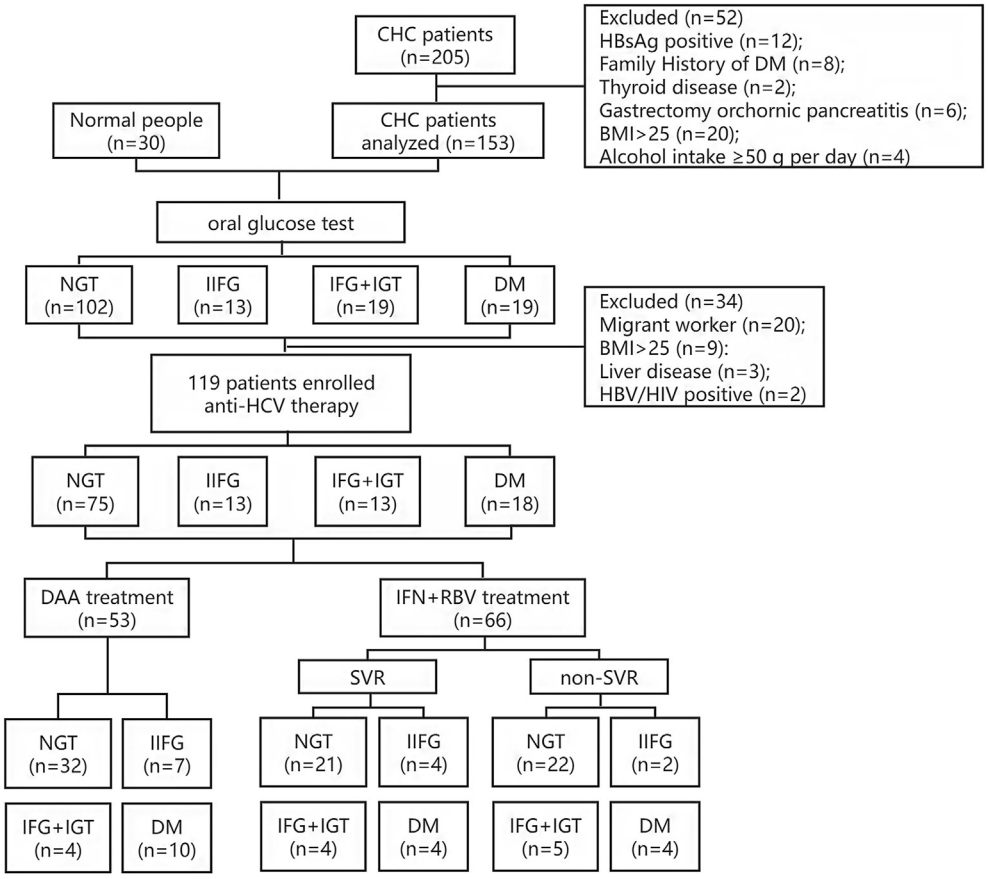

Fig. 1. The flowchart of patient analysis. One hundred fifty-three CHC patients and thirty normal people with normal fasting glucose levels were studied. Of these, 119 CHC patients were enrolled in the HCV therapy study. NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IIFG, isolated IFG; IFG + IGT, IFG and IGT combined; DM, diabetes.

24 weeks in patients with HCV genotype 2/3 and for 48 weeks in those with HCV genotype non-2/3 according to the AASLD guidelines (Panel, 2018). In addition, all patients received daily oral ribavirin (Rebetol; Schering Plough Corp., USA) and RBV dosage was determined by body weight according to HCV genotype in both PEG-IFN groups: 800 mg/day for genotype 2/3 and 1000 mg/day (body weight ≤75 kg) or 1200 mg/day (>75 kg) for genotype non 2/3 (Panel, 2018). Patients undergoing DAA treatment got a fixed-dose combination pill containing 400 mg of sofosbuvir and 90 mg of ledipasvir once daily. HCV treatment outcomes were evaluated in accordance to SVR, defined as HCV RNA negativity 12 weeks or more post-treatment (Panel, 2018). Non-responders and patients who relapsed were included in the patients who did not achieve SVR (non-SVR). Six months following the conclusion of the treatment, patients who had received HCV therapy with or without SVR underwent a second round of metabolic testing.

The study involved 30 participants who underwent physical examinations at the First Affiliated Hospital of Xinjiang Medical University's Health Management Center between January 2014 and January 2019. Each individual underwent an investigation that included a questionnaire, physical examination, abdominal ultrasound, and testing of blood biochemical indicators. All participants exhibited normal indicators and had no history of alcohol consumption excluded from severe cardiocerebrovascular disease, chronic viral hepatitis, genetic liver disease, drug-induced liver disease, autoimmune liver disease, or abnormal renal function.

2.2. Metabolic testing

All participants underwent oral glucose tolerance (OGTT) with 75 g of glucose according to the recommendations of the National Diabetes Data Group of the National Institutes of Health. Patients fasted overnight and blood samples were drawn for the determination of plasma glucose, insulin, and C-peptide levels before and at 30, 60, 120, and 180 min after glucose loading. Plasma glucose concentrations were measured by the glucose oxidase method. The concentrations of insulin and C-peptide were quantified with a human Metabolism Multiplex Assay using a FLEXMAP 3D quantification system (Luminex, Austin, TX). Using the 2003 American Diabetes Association criteria (Genuth et al., 2003), Glucose tolerance was evaluated as follows: NGT [fasting plasma glucose (FPG) < 5.6 mmol/L and 2 h plasma glucose (PG) < 7.8 mmol/L], isolated impaired fasting glucose (IIFG) (FPG 5.6–6.9 mmol/L and 2 h PG < 7.8 mmol/L), isolated impaired glucose tolerance (IGT) (FPG < 5.6 mmol/L and 2 h PG between 7.8 and 11.0 mmol/L), IFG + IGT (FPG 5.6–6.9 mmol/L and 2 h PG 7.8–11.0 mmol/L), and DM subjects (FPG ≥ 7.0 mmol/L and/or 2 h PG ≥ 11.1 mmol/L).

2.3. Insulin resistance and β-cell function

Insulin resistance was assessed by the homeostatic model assessment-insulin resistance (HOMA-IR) score. Insulin sensitivity derived from the OGTT was estimated as proposed by Matsuda and DeFronzo (Matsuda insulin sensitivity index, Matsuda ISI) (Stancakova et al., 2009). The composite (Matsuda) ISI is an index of whole-body insulin sensitivity and highly correlates with the rate of whole-body glucose disposal during the euglycaemic insulin clamp test (Matsuda and DeFronzo, 1999). Insulin secretion derived from the fasting state was calculated as HOMA-β. Insulin secretion indexes derived from the OGTT were calculated as the insulinogenic index (IGI_{30}) (Herzberg-Schafer et al., 2010), early C-peptide response (ΔC-peptide$_{30}$) (Iwasaki et al., 1978) and the disposition index (DI, a gold standard for measuring β-cell function) (Herzberg-Schafer et al., 2010). The IGI is a marker of early insulin secretion and correlates well with the acute insulin response to an intravenous glucose load (Herzberg-Schafer et al., 2010), whereas the ΔC-peptide$_{30}$ after a glucose load is a marker of pancreatic insulin secretion in liver disease (Iwasaki et al., 1978; Kosaka et al., 1996; Seltzer et al., 1967). Total insulin and C-peptide secretion levels were calculated as the total area under the response curve 2 h after OGTT adjusted for the corresponding glucose AUC ($InsAUC_{120}/GluAUC_{120}$ and C-peptide $AUC_{120}/GluAUC_{120}$). We also calculated three disposition indexes (DI_{30}, DI_{60} and DI_{120}) as a measure of the β-cell response to insulin sensitivity because these indexes have been shown to predict the conversion to diabetes for evaluating β-cell function (Defronzo, 2009; Lorenzo et al., 2010; Ohn et al., 2016). In a previous study analyzing several measures of disposition indexes based on insulin secretion and insulin sensitivity from an OGTT, the product of InsAUC/GluAUC and Matsuda ISI was the only combination that followed the hyperbolic relationship that is the requirement for a disposition index (Retnakaran et al., 2008). All the indexes used for the evaluation of insulin resistance and β-cell function are described in Supplementary Table S1.

2.4. Statistical analysis

The baseline clinical characteristics of patients are expressed as mean (standard deviation), median (range), or frequencies. Statistical comparisons between the two groups were made using $\chi 2$ tests for categorical variables and *t*-tests for continuous variables, and continuous variables were compared across the categories of glucose tolerance by ANOVA. Pairwise comparisons between the groups were performed by Bonferroni post hoc tests (with *P* value adjustment for multiple tests for each variable). Statistical analyses were performed using GraphPad Prism Ver. 6.01 (San Diego, CA, USA). Coefficients were calculated from the linear discriminant function of the variables. The selection of variables was based on a stepwise regression analysis using the forward selection method. A multivariate logistic regression analysis was used to identify the factors significantly associated with SVR (dependent variables coded as 0 = absent or 1 = present). Variables significantly associated with the dependent variable in the univariate analysis were included in the multivariate logistic regression model at first and removed if not significantly associated with the dependent variable at the *P*-value of 0.05. Analyses were performed using SPSS 17.0 (IBM, IL, USA) and GraphPad Prism Ver. 6.01 (San Diego, CA, USA). Differences were considered significant at $P < 0.05$ (*, $P < 0.05$).

3. Results

3.1. Patient characteristics

A total of 153 subjects were enrolled in the study. Among the study participants, 66% were found to have normal glucose tolerance (NGT), while 21% were diagnosed with pre-diabetes and 13% had diabetes (DM). Out of all the subjects, 12 individuals (8%) had isolated IFG (IIFG), whereas 19 individuals (13%) had IFG and IGT combined. Interestingly, none of the subjects exhibited isolated IGT (IIGT). The patient characteristics including age, sex, and BMI were similar across different categories of glucose metabolism (Table 1). However, statistically significant differences among the groups were observed in terms of HCV-related factors such as viral load ($P = 0.002$) and genotype ($P = 0.008$) (Table 1).

3.2. Glucose and insulin responses to oral glucose

Glycemic control measurements varied according to their classification among the five groups of glucose metabolism. During OGTT, although the glucose levels were higher at each timepoint, the pattern of glucose response in IFG was similar to that of the NGT group and returned to normal at 120 min. However, those with combined IFG/IGT had persistently elevated glucose levels and did not return to normal at the 120-min timepoint, similar to individuals with DM (Fig. 2A–C).

3.3. Insulin sensitivity and β-cell function

When comparing insulin resistance indices, Matsuda ISI showed a significant decrease from 79.57 ± 11.86 ($P < 0.0001$) in NGT to 40.59 ±

Table 1
Clinical and anthropometric characteristics of CHC patients according to glucose tolerance status.

Parameter		Control	HCV				*P* value
			NGT	IIFG	IFG + IGT	DM	
Number of patients		30	102	13	19	19	
Age		35 ± 7	38 ± 7	39 ± 3	41 ± 7	41 ± 6	0.022*
Sex; M/F		15/15	63/39	7/6	7/12	10/9	0.317
BMI (kg/m^2)		22.1 ± 1.2	23.1 ± 1.7	23.2 ± 1.4	22.6 ± 1.6	22.5 ± 1.2	0.291
HCV genotype	2a/1b		7	0	4	0	0.008*
	2a		32	6	6	14	
	1b		63	7	9	5	
HCV viral load	low		82	8	13	9	0.002*
	mild		15	2	1	0	
	high		5	3	5	10	

Notes: Data are means ± SD or number (%). *P* values for overall comparison between five categories of glucose tolerance are shown (ANOVA for continuous variables, χ^2 test for categorical variables). Differences were considered significant at $P < 0.05$ (*, $P < 0.05$).

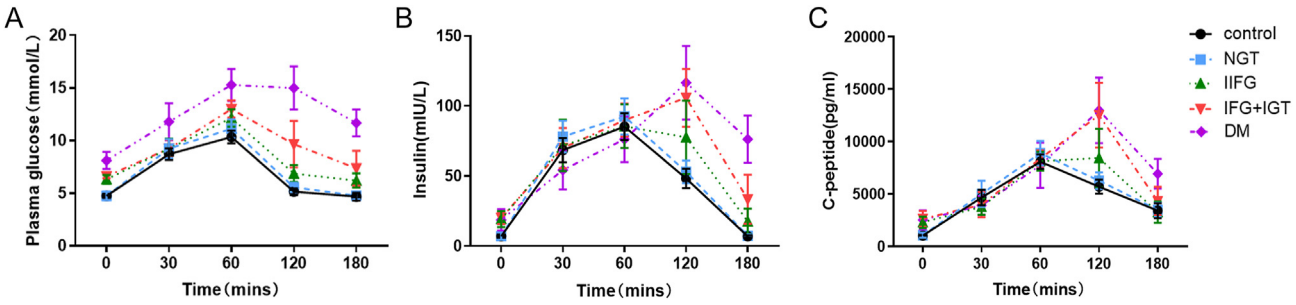

Fig. 2. The glucose (A) insulin (B) and C-peptide (C) levels after an oral load of 75 g glucose in healthy control (○) or CHC patients with DM (◇), IFG + IGT (▽), IIFG (Δ) and NGT (□). Data are presented as the mean ± SD.

8.65 ($P < 0.0001$) in isolated IFG, further decreasing to 34.07 ± 5.75 ($P < 0.0001$) in combined IFG/IGT and to 31.56 ± 10.49 ($P < 0.0001$) in DM. A significantly greater increase in isolated IFG than in NGT (5.44 ± 1.71 vs. 1.71 ± 0.62, $P < 0.0001$) was also found when insulin sensitivity was assessed with HOMA-IR, which further increased in IFG + IGT and DM to 5.90 ± 1.28 ($P < 0.0001$) and 6.76 ± 2.88 ($P < 0.0001$), respectively (Table 2, Supplementary Fig. S1 A and B).

The early-phase insulin release IGI_{30} decreased progressively from 7.62 ± 1.31 in individuals with NGT, to 5.84 ± 2.47 in isolated IFG, further decreasing to 5.38 ± 1.19 in IFG + IGT and reaching the lowest level of 3.06 ± 1.26 in DM. Similarly, another marker of early-phase insulin release, $\Delta C\text{-peptide}_{30}$, showed a significant decline from NGT (423.68 ± 132.79) to isolated IFG (165.10 ± 54.48, $P < 0.0001$), IFG + IGT (152.7 ± 138.5, $P < 0.0001$) and diabetes (119.68 ± 66.75, $P < 0.0001$). Another pronounced decrease was observed in isolated IFG compared to NGT when assessing early-phase insulin release using DI_{30}, with values dropping from 489.83 ± 73.61 to 238.68 ± 81.84 ($P < 0.0001$). DI_{30} was also reduced to 189.67 ± 26.97 in the IFG + IGT and to 112.74 ± 33.09 in DM subjects (Table 2, Supplementary Fig. S1 C and E). DI decreased significantly from 2.04 ± 0.53 in NGT, to 1.16 ± 0.65 ($P < 0.0001$) in isolated IFG, and further declined to 1.09 ± 0.45 ($P < 0.0001$) in IFG + IGT and 0.83 ± 0.75 ($P < 0.0001$) in DM (Table 2 and Supplementary Fig. S1F). Furthermore, the overall insulin response to oral glucose ($InsAUC_{120}/GluAUC_{120}$ and $C\text{-peptideAUC}_{120}/GluAUC_{120}$) exhibited no significant change in pre-diabetic states compared to the NGT group (Table 2, Supplementary Fig. S1 G and H). However, there was a significant decrease in total DI_{120} to 308.30 ± 74.22 ($P < 0.0001$) in isolated IFG, to 260.0 ± 36.68 ($P < 0.0001$) in IFG ± IGT, and to 169.89 ± 54.35 ($P < 0.0001$) in diabetes when compared with NGT (641.35 ± 89.20) (Table 2 and Supplementary Fig. S1I). The decline observed in Matsuda ISI and DIs scores suggests that impaired β-cell compensation plays a crucial role in the progressive deterioration of insulin sensitivity leading to impaired glucose tolerance following HCV infection.

Stepwise forward multiple linear regression analyses revealed HOMA-IR ($P = 0.009$), Matsuda ISI ($P = 0.027$), IGI_{30} ($P < 0.001$), $\Delta C\text{-peptide}_{30}$ ($P = 0.003$), $InsAUC_{120}/GluAUC_{120}$ ($P < 0.001$), $C\text{-peptideAUC}_{120}/GluAUC_{120}$ ($P < 0.001$), DI ($P = 0.007$), DI_{30} ($P < 0.001$), and DI_{120} ($P = 0.001$) were significantly correlated with HCV viral load. HOMA-IR ($P = 0.025$), Matsuda ISI ($P = 0.008$), DI ($P = 0.013$), DI_{30} ($P = 0.02$), DI_{120} ($P = 0.021$) also exhibited significant associations with age (Supplementary Table S2).

3.4. Cohort HCV therapy characteristics

The study enrolled a total of 119 CHC patients who received anti-HCV therapy. Among them, DAA combinations were administered to 53 patients, all of whom achieved SVR. After a follow-up period of six months, SVR was observed in 33 (50%) patients who underwent PEG-IFN and ribavirin (IFN + RBV) treatment. The characteristics of the patients were comparable across different treatment groups (Supplementary Table S3). In the IFN + RBV therapy group, viral load and host variables such as age, gender, BMI, HCV RNA levels, fasting glucose levels, fasting insulin levels, and fasting C-peptide levels showed no significant differences between participants who achieved SVR and those who did not (Supplementary Table S4).

Comparison of pre- and post-treatment values in HCV patients who achieved SVR revealed a decrease in mean FPG from 5.78 ± 1.65 mmol/L before therapy to 4.68 ± 0.39 mmol/L ($P < 0.0001$) after DAA treatment (12 weeks after discontinuation of therapy). Similarly, subjects undergoing IFN + RBV treatment and achieving SVR showed a trend of reduced FPG levels (5.58 ± 1.24 mmol/L vs. 4.74 ± 0.30 mmol/L, $P = 0.0004$). However, there was no significant difference in mean FPG for subjects who underwent antiviral treatment but did not achieve SVR initially at the 24-week follow-up (5.16 ± 1.01 mmol/L vs. 5.33 ± 1.02 mmol/L, $P = 0.51$). A significant decrease in serum fasting insulin levels was observed from 12.10 ± 6.70 mIU/L to 7.29 ± 0.93 mIU/L ($P < 0.0001$) after DAA treatment and also decreased from 12.83 ± 6.88 mIU/L to

Table 2
β-Cell function and insulin resistance indices in CHC patients or controls from the fasting or OGTT state.

Parameter	Control	HCV				
		NGT	IIFG	IFG + IGT	DM	*P* value
n	30	102	13	19	19	
β-cell function						
Fasting glucose (mmol/l)	4.76 ± 0.39	4.68 ± 0.41	6.32 ± 0.41[a b]	6.62 ± 0.21[a b c]	8.11 ± 0.80[a b c d]	<0.0001
Fasting insulin (mIU/l)	7.06 ± 0.88	8.19 ± 2.68	19.26 ± 5.70[a b]	20.01 ± 4.28[a b]	18.68 ± 7.67[a b]	<0.0001
Fasting C-peptide (pg/ml)	1019.41 ± 90.12	1060.51 ± 230.97	2289.15 ± 542.8[a b]	2623.36 ± 759.9.54[a b]	2530.96 ± 905.49[a b]	<0.0001
HOMA-β	124.62 ± 47.02	157.57 ± 80.47	137.1 ± 38.91	128.2 ± 28.01	82.12 ± 33.93	0.001
IGI_{30}	13.00 ± 2.19	7.62 ± 1.31[a]	5.84 ± 2.47[a b]	5.38 ± 1.19[a b c]	3.06 ± 1.26[a b c d]	<0.0001
$\Delta C\text{-peptide}_{30}$	418.97 ± 85.27	423.68 ± 132.79	165.10 ± 54.48[a b]	152.7 ± 138.5[a b]	119.68 ± 66.75[a b]	<0.0001
$InsAUC_{120}/GluAUC_{120}$	7.82 ± 0.77	8.11 ± 0.81	7.67 ± 1.19	7.71 ± 0.92	5.51 ± 1.07[a b c d]	<0.0001
$C\text{-peptideAUC}_{120}/GluAUC_{120}$	724.41 ± 55.81	740.6 ± 66.84	603.8 ± 87.95	728.9 ± 92.59	553.78 ± 109.62[a b d]	<0.0001
Disposition index						
DI	2.25 ± 0.38	2.04 ± 0.53	1.16 ± 0.65[a b]	1.09 ± 0.45[a b]	0.83 ± 0.75[a b]	<0.0001
DI_{30}	501.38 ± 58.25	489.83 ± 73.61	238.68 ± 81.84[a b]	189.67 ± 26.97[a b]	112.74 ± 33.09[a b c d]	<0.0001
DI_{120}	698.52 ± 63.5	641.35 ± 89.20	308.30 ± 74.22[a b]	260.0 ± 36.68[a b]	169.89 ± 54.35[a b c d]	<0.0001
Insulin resistance						
Matsuda ISI	89.78 ± 9.16	79.57 ± 11.86[a]	40.59 ± 8.65[a b]	34.07 ± 5.75[a b]	31.56 ± 10.49[a b]	<0.0001
HOMA-IR	1.49 ± 0.19	1.71 ± 0.62	5.44 ± 1.71[a b]	5.90 ± 1.28[a b]	6.76 ± 2.88[a b]	<0.0001

Notes: Data are means ± SD. Bonferroni post hoc test: all pairwise comparisons between categories of glucose tolerance were marked as follows: a, $P < 0.05$ control vs. NGT, IIFG, IFG + IGT, DM; b, $P < 0.05$ NGT vs. IIFG, IFG + IGT, DM; c, $P < 0.05$ IIFG vs. IFG + IGT, DM; d, $P < 0.05$ IFG + IGT vs. DM. NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IIFG, isolated IFG; IFG + IGT, IFG and IGT combined; DM, diabetes.

7.18 ± 0.96 mIU/L ($P < 0.0001$) at 6 months after discontinuation of IFN + RBV therapy in those with SVR; however, there was no significant change observed in those without SVR (Table 3).

To gain a better understanding of insulin sensitivity and beta cell function in patients who underwent anti-HCV therapy, we recorded glucose and insulin data at 30, 60, and 120 min after the OGTT. Following DAA treatment, there was a significant decrease in 2-h plasma glucose levels during OGTT from 8.27 ± 3.93 mmol/L to 5.01 ± 0.53 mmol/L ($P < 0.0001$), as well as a reduction from 7.02 ± 2.95 mmol/L to 4.68 ± 0.29 mmol/L ($P < 0.0001$) after IFN + RBV therapy in those with SVR (Table 3). Additionally, DAA treatment resulted in a significant decrease in mean HOMA-IR value (3.94 ± 2.27 vs. 1.31 ± 0.84, $P < 0.0001$) and an increase in Matsuda ISI score (62.16 ± 24.57 vs. 115.46 ± 11.24, $P < 0.0001$) respectively (Table 3, Supplementary Fig. S2A and B). SVR patients also showed improvement in HOMA-IR (3.41 ± 2.31 vs. 1.52 ± 0.25, $P < 0.0001$) and Matsuda ISI score (61.71 ± 23.98 vs. 116.05 ± 11.03, $P < 0.0001$) during IFN + RBV therapy (Table 3, Supplementary Fig. S3A and B). There were no significant changes observed between pre- and post-treatment regarding insulin secretion levels measured by HOMA-β (Supplementary Fig. S2C and Fig. S3C).

However, DAA treatment led to remarkable improvements in IGI_{30} (6.31 ± 2.10 vs. 7.35 ± 0.86, $P < 0.0001$) and DI (1.66 ± 0.78 vs. 1.95 ± 0.36, $P = 0.0168$) scores during OGTT (Table 3 and Supplementary Fig. S2D and E). Similarly, IGI_{30} (6.73 ± 2.35 vs. 7.99 ± 0.92, $P = 0.0059$) and DI (1.65 ± 0.80 vs. 2.32 ± 0.43, $P < 0.0001$) scores of SVR patients were also significantly improved after IFN + RBV treatment (Supplementary Fig. S3D and E). We specifically observed improved DI_{30} and DI_{120} scores following viral clearance, suggesting enhanced β-cell function (Table 3, Supplementary Fig. S2F and G, Supplementary Fig. S3F and G). Insulin resistance assessed by HOMA-IR and Matsuda ISI indices, as well as β-cell function assessed by IGI_{30}, DI, DI_{30} and DI_{120} scores exhibited no significant change during IFN + RBV therapy without achieving SVR (Table 3).

In addition, based on the thresholds presented in the NGT group (Table 2), a significant proportion of patients treated with DAA demonstrated a transition from abnormal to normal baseline levels for HOMA-IR (72%, 37/51) (Fig. 3A). Matsuda ISI levels showed normalization from abnormal to normal baseline in all patients (100%, 32/32) (Fig. 3B). Furthermore, improvements were observed in IGI_{30} and DI levels, reaching normal baseline values in approximately 41% (15/38) and 40%

Table 3
β-Cell function and insulin resistance in CHC patients before and after anti-HCV therapy.

Parameter	DAA treatment			IFN + RBV treatment					
	SVR			SVR			Non SVR		
	Pre treatment	Post treatment	*P* value	Pre treatment	Post treatment	*P* value	Pre treatment	Post treatment	*P* value
$Log_{10}HCV$ RNA	4.61 ± 1.95	N.D.		4.39 ± 1.16	N.D.		4.48 ± 1.16	5.01 ± 0.83	0.11
Fasting glucose (mmol/l)	5.78 ± 1.65	4.68 ± 0.39	<0.0001	5.58 ± 1.24	4.74 ± 0.30	0.0004	5.16 ± 1.01	5.33 ± 1.02	0.51
Fasting insulin (mIU/l)	12.10 ± 6.70	7.29 ± 0.93	<0.0001	12.83 ± 6.88	7.18 ± 0.96	<0.0001	11.34 ± 7.22	12.59 ± 7.15	0.48
1 h glucose (mmol)	12.07 ± 1.82	5.31 ± 0.39	<0.0001	11.71 ± 1.57	5.23 ± 0.41	<0.0001	10.54 ± 2.76	11.25 ± 0.99	0.16
2 h glucose (mmol/l)	8.27 ± 3.93	5.01 ± 0.53	<0.0001	7.02 ± 2.95	4.68 ± 0.29	<0.0001	6.34 ± 2.34	7.18 ± 2.82	0.19
HOMA-β	138.4 ± 90.59	133.7 ± 40.69	0.72	138.0 ± 51.45	120.9 ± 28.33	0.101	148.19 ± 61.28	140.98 ± 34.34	0.55
IGI_{30}	6.31 ± 2.10	7.35 ± 0.86	<0.0001	6.73 ± 2.35	7.99 ± 0.92	0.0059	6.75 ± 1.84	6.61 ± 1.92	0.75
DI	1.66 ± 0.78	1.95 ± 0.36	0.0168	1.65 ± 0.80	2.32 ± 0.43	<0.0001	1.75 ± 0.73	1.62 ± 0.65	0.41
DI_{30}	361.78 ± 176.25	691.98 ± 81.51	<0.0001	376.57 ± 172.75	732.54 ± 85.71	<0.0001	445.38 ± 177.09	411.80 ± 162.69	0.42
DI_{120}	480.41 ± 223.19	749.91 ± 91.42	<0.0001	492.32 ± 211.35	804.0 ± 85.76	<0.0001	580.03 ± 213.70	535.64 ± 204.30	0.39
HOMA-IR	3.94 ± 2.27	1.31 ± 0.84	<0.0001	3.41 ± 2.31	1.52 ± 0.25	<0.0001	2.85 ± 2.43	3.27 ± 2.48	0.49
Matsuda ISI	62.16 ± 24.57	115.46 ± 11.24	<0.0001	61.71 ± 23.98	116.05 ± 11.03	<0.0001	75.16 ± 27.63	68.82 ± 24.08	0.32

Notes: Data are means ± SD. *P* values are based on the Paired-samples *t*-test. *$P < 0.05$. N. D., not detected.

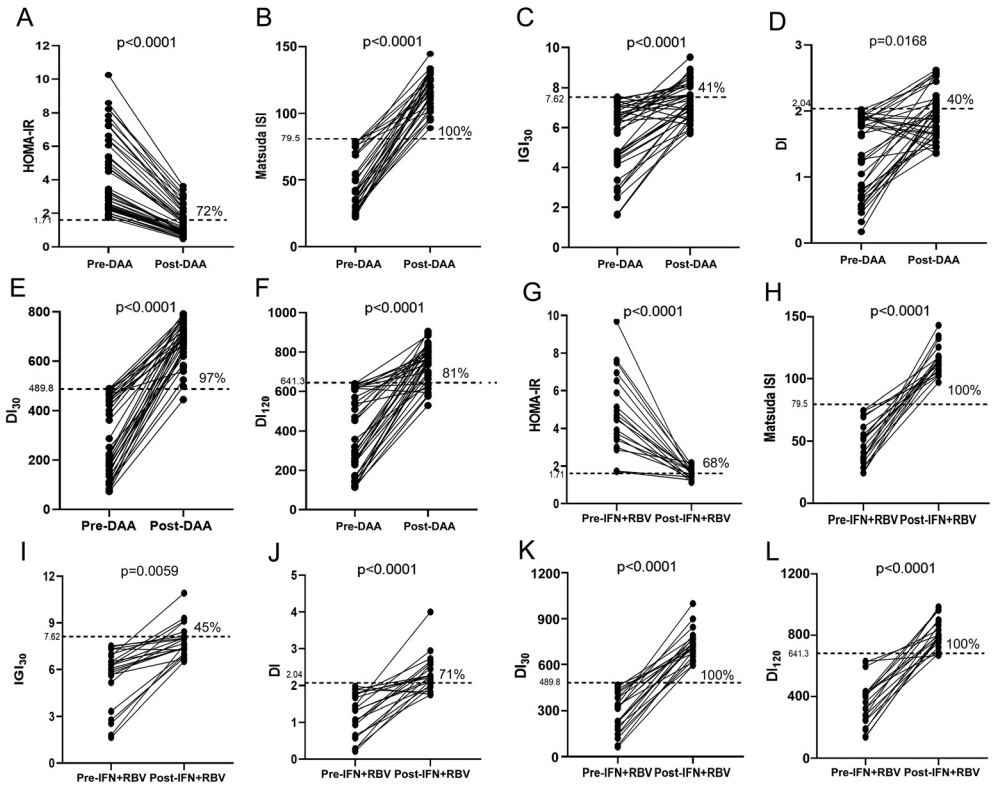

Fig. 3. HOMA-IR (A), Matsuda ISI (B), IGI_{30} (C), DI (D), DI_{30} (E) and DI_{120} (F) levels in HCV patients before and after DAA treatment. HOMA-IR (G), Matsuda ISI (H), IGI_{30} (I), DI (J), DI_{30} (K) and DI_{120} (L) levels in HCV patients who achieved SVR before and after IFN + RBV treatment. Cutoff values were applied as indicated.

(15/39) of patients respectively (Fig. 3C and D). Similarly, DI_{30} and DI_{120} levels improved to normal baseline in the majority of patients treated with DAA; specifically, this was observed in approximately 97% (37/38) and 81% (31/38) of cases respectively (Fig. 3E and F). Additionally, among those treated with IFN + RBV after SVR, 68% (13/19) of patients exhibited normalization for HOMA-IR and 100% of patients exhibited normalization for Matsuda ISI levels (Fig. 3G and H) while IGI_{30} and DI levels normalized in approximately 45% (10/22) and 71% (15/21) of cases, respectively (Fig. 3I and J). DI_{30} and DI_{120} levels showed normalization from abnormal to normal baseline in all patients (100%, 32/32) (Fig. 3K and L). Thus, viral eradication demonstrated a favorable impact on insulin sensitivity and β-cell function in these patients, irrespective of fasting or OGTT state. Univariate analysis revealed that SVR was not associated with β-cell function as assessed by HOMA-β, IGI_{30}, and DIs following HCV infection in the entire population (Supplementary Table S5).

4. Discussion

T2DM and HCV infection are closely associated. Uncertainty surrounds the function of dysfunctional β-cells. In this study, we utilized validated surrogate markers to assess insulin release in CHC patients, focusing on β-cell function during the conversion from NGT to T2DM. Our findings indicate that both early phase and total insulin secretion were lower in individuals with pre-diabetic states compared to those with NGT. Furthermore, we directly evaluated β-cell function in a cohort of HCV patients and examined its correlation with HCV treatment outcomes. The dysfunction of β-cell was addressed through the achievement of SVR by IFN/RBV or DAA therapy. However, the accomplishment of SVR was not significantly correlated with β-cell dysfunction.

In this study, we observed high rates of pre-diabetes in our HCV-infected population, which is consistent with the rates found in the general population. Previous studies conducted on individuals without HCV infection have identified increasing age and body mass index (BMI), sex, waist-to-hip ratio, and fat mass as risk factors for pre-diabetes (Stancakova et al., 2009). However, in our HCV-infected population, age and sex were not associated with pre-diabetes. These findings suggest that there may be a distinct phenotype of pre-diabetes among individuals infected with HCV.

The pre-diabetic state, as defined by the OGTT, encompasses IFG, IGT, or their combination (Genuth et al., 2003). In contrast to studies conducted on HCV-uninfected individuals, CHC patients in the pre-diabetic state exhibited isolated IFG rather than isolated IGT. Epidemiological studies have shown that IFG and IGT represent two distinct subgroups of abnormal glucose tolerance (Carnevale Schianca et al., 2003; Nathan et al., 2007) and are likely to have different pathophysiological mechanisms. Previous reports suggest that the coexistence of hepatic insulin resistance and defective insulin secretion in isolated IFG leads to excessive fasting hepatic glucose production contributing to fasting hyperglycemia (Nathan et al., 2007). Consistently, our previous findings demonstrated an exacerbation of gluconeogenesis in HCV infection (Chen et al., 2015). Our results imply that the development of HCV-associated T2DM occurs via the elevation of FPG levels. Furthermore, we observed significantly lower early-phase insulin release (IGI_{30}) and DI_{30} (a measure of β-cell compensation for the insulin sensitivity) in both isolated IFG and IFG + IGT subjects compared to NGT subjects. This suggests impaired β-cell function, particularly dysfunction in early-phase insulin secretion may exist following HCV infection within the range of FPG impairment among CHC patients. Importantly, DI_{120}, a total β-cell response to insulin sensitivity was also lower in pre-diabetic CHC patients, despited the unchanged total insulin release. These findings indicate that insulin resistance and impairment of β-cell function in CHC patients is predictive of an underlying conversion to diabetes in pre-diabetic CHC patients, which can help to identify patients with abnormal glucose metabolism more accurately and earlier, and prevent or delay the risk of diseases such as type 2 diabetes through timely intervention.

The precise mechanism underlying the complex interaction between host and viral factors that leads to impaired glycemic control in CHC

patients remains unknown. Several studies suggest that HCV disrupts glucose homeostasis by interfering with insulin signaling through mechanisms that may be genotype-specific and influenced by higher levels of viral replication (Hsu et al., 2008). The precise nature of the complex interaction of host and viral factors that leads to impaired glycemic control in CHC patients is unknown. Consistently, this study found that viral factors, including HCV viral load, duration of infection, and genotype were significantly associated with pre-diabetes and diabetes. Moreover, pharmacological treatment resulted in improved β-cell function indices. The possible cause for this phenomenon could be attributed to the infection of HCV in extrahepatic tissue, including pancreatic cells. HCV RNA replication has been observed in non-hepatic cells, although the replication efficiency is rather low (Dreux et al., 2012; Fletcher et al., 2010). Additionally, HCV RNA was detected in pancreatic tissue harvested from CHC patients (Masini et al., 2005). Recent reports indicate that brain microvascular endothelial cells express all essential receptors for HCV entry and human beta cells possess all necessary factors for HCV entry as well. These findings raise the possibility that HCV may employ different mechanisms, such as other receptor molecules that mediate the entry and replication of HCV in the extrahepatic tissue, including pancreatic cells, and directly cause impairment of β-cell function.

5. Conclusions

Overall, our study demonstrates that in addition to insulin resistance, β-cell dysfunction is observed in pre-diabetic states of chronic HCV infection. Pancreatic β-cell dysfunction may occur in chronic HCV even without advanced fibrosis or liver disease progression. Therefore, HCV therapy in these individuals may be warranted. Compared to HCV therapy that fails to achieve SVR, achieving SVR through HCV therapy significantly enhances insulin secretion, reverses insulin resistance, improves glycemic control and has the potential to prevent diabetes-related clinical manifestations and complications.

Data availability

The datasets analyzed in this study are available from the corresponding authors on reasonable request.

Ethics statement

Prior to enrolment, each participant in this study provided written informed consent. The methods and experimental protocols of the study, as well as the use of all patients' and/or their relatives' clinical and pathological information for research, were approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (No.S-7). All patients and/or their relatives gave written informed consent for this information to be used for research and stored in the hospital database. Patients with comorbid conditions that precluded inclusion in the study (such as chronic HBV, elevated BMI, and liver disease) were offered treatment for their chronic HCV at our hospital. All study procedures were carried out in compliance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, as well as the ethical guidelines established by our institution's research committee. The methods were applied in compliance with the accepted regulations.

Author contributions

Jizheng Chen: investigation, data curation, writing-original draft, supervision. Pan Qiu: investigation, data curation. Tingfeng Zhao: investigation. Haowei Jiang: data curation, methodology. Kebinur Tursun: conceptualization. Sulaiman Ksimu: conceptualization. Xinwen Chen: conceptualization, writing-review&editing, supervision. Qian Wang: conceptualization, data curation, writing-review&editing, supervision.

Conflict of interest

Prof. Xinwen Chen is an editorial board member for *Virologica Sinica* and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2024.06.007.

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