https://doi.org/10.31925/farmacia.2022.4.9 **ORIGINAL ARTICLE**

CHANGES IN BIOCHEMICAL PARAMETERS IN DIABETES AFTER SUCCESSFUL DIRECT-ACTING ANTIVIRAL TREATMENT OF HEPATITIS C VIRUS

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Abstract

Diabetes Mellitus (DM) and Chronic Hepatitis C Virus (HCV) have a strong interconnection in general practice. Recent studies have correlated insulin resistance risk with liver dysfunction. Haptoglobin is a biomarker that is modified in liver disease and is involved in metabolic diseases. Our study aims to investigate the changes in haptoglobin and bilirubin levels after direct-acting antiviral (DAA) treatment for viral hepatitis C in relation to diabetes status and hypertension in order to identify potential biomarkers for the prognosis of liver disease in diabetic patients after obtaining sustained virological response (SVR). Eighty patients (40 with Type 2-DM (T2DM) and 40 without T2DM) treated with DAAs for HCV infection that obtained SVR were monitored for biochemical markers before and 3 years after treatment. We report that HCV clearance improves not only the biomarkers of liver function but also TB and Hp, which are strongly connected with insulin resistance in T2DM, leading to better control of blood glucose levels, obesity, and lipid metabolism. These two biomarkers should be further investigated not only to understand better the physiopathological pathways by which they affect TD2M progression, but also how their levels might be raised in the pursuit of better control of diabetes, cardiovascular complications, and other diseases.

Rezumat

Diabetul zaharat (DZ) și hepatita C (HC) se află într-o strânsă interconexiune în practica medicală. Studii recente au corelat rezistența la insulină cu disfuncția hepatică. Haptoglobina este un biomarker ale cărei valori se modifică în afecțiuni hepatice și este implicată în tulburări metabolice. Studiul își propune să investigheze modificările haptoglobinei și bilirubinei după tratamentul hepatitei C cu antivirale directe, corelat cu statusul patologiei diabetice și hipertensive, în vederea identificării unor biomarkeri capabili să ofere informații despre evoluția funcției hepatice la pacienții diabetici după obținerea unui răspuns viral optim (RVO). Optzeci de pacienți (40 cu diabet zaharat de tip 2 și 40 fără diabet zaharat de tip 2) tratați cu antivirale directe pentru hepatita C care au obținut RVO au fost monitorizați timp de 3 ani de la începerea acestuia. S-a observat că o rată mai mare de vindecare a hepatitei C îmbunătățește nu doar biomarkerii funcției hepatice, dar și bilirubina totală și haptoglobina care sunt puternic corelate cu rezistența la insulină, conducând la un mai bun control al glicemiei, obezității și metabolismului lipidic. Acești biomarkeri trebuie să fie investigați în continuare, pentru o mai bună înțelegere a fiziopatologiei diabetului zaharat de tip II și a evoluției acestuia.

Keywords: HCV, type 2 diabetes, total bilirubin, haptoglobin

Introduction

Chronic hepatitis C virus (HCV) is a major cause of chronic liver disease, and the long-term impact varies from minimal changes to extensive fibrosis, cirrhosis and even progression to hepatocellular carcinoma (HCC) [1]. The goal of chronic HCV infection treatment is to achieve a sustained virological response (SVR) stable over time in order to reduce morbidity and mortality [2]. Diabetes Mellitus (DM) is one of the most prevalent chronic metabolic disorders reported in the world. Type 2 DM (T2DM) accounts for 90 - 95% of all

diabetic cases. Several risk factors, including obesity, ageing genetic predisposition and viral infections, have been associated with T2DM. Among viral infections, HCV infection has become a great concern [3]. Nearly three-quarters of patients with HCV infection also suffer from extrahepatic manifestations, T2DM being one of the most common [4].

The connection between DM and liver failure has been studied in the past years. DM is linked with liver cirrhosis. One possible pathway could be oxidative stress that is involved in the generation of DM through improper insulin action [5, 6]. DM is also associated with hepatocellular and cardiovascular diseases. Lately, two serum markers came into focus as they are presumably associated with diabetes incidence and progression: bilirubin and haptoglobin.

The association between bilirubin levels and DM incidence, progression and prognosis has become a focus of researchers in the last years. Previously, a variety of proofs strengthened the association between serum bilirubin levels and the incidence of diabetes complications. Traditionally, serum bilirubin has for a long time been considered a sign of liver dysfunction [7].

Haptoglobin (Hp) was originally discovered in human plasma and is considered to be a reactive protein in the acute inflammatory phase. Hp gene has two alleles: Hp1 and Hp2, and 3 genotypes (Hp 1-1, Hp 1-2 and Hp 2-2), each linked to different diseases [8]. In addition to metabolic diseases, Hp is also associated with many other pathologies such as respiratory diseases, cardiovascular and autoimmune diseases. As an acute phase protein, Hp increases rapidly in serum after infection and inflammation [9]. The expression level and gene polymorphism of Hp is considered to be implicated in the occurrence and development of metabolic diseases like obesity, type 2 diabetes (T2DM), complications of diabetes and others [10].

Our study aimed to investigate the changes in haptoglobin and bilirubin levels after direct-acting antiviral (DAA) treatment for viral hepatitis C in relation to diabetes status and hypertension in order to identify potential biomarkers for prognosis of liver disease in diabetic patients after obtaining sustained virological response (SVR).

Materials and Methods

Patients and biochemical exam

For our study, we selected 80 patients that were infected with HCV, equally divided between those that presented T2DM and those without T2DM. HCV genotype was determined in all patients as genotype 1b in compliance with the treatment protocol. Every patient received ombitasvir 12,5 mg/paritaprevir 75 mg/ritonavir 50 mg (Viekirax®) 2 pills/day (AbbVie, USA) and dasabuvir 250 mg (Exviera®) 2 pills/day for 8 weeks (AbbVie, USA). All patients included in the study obtained at 12 weeks after treatment SVR defined as negative HCV RNA. All patients had an initial biochemical exam done before being admitted to the DAA treatment program, weight and height were measured to establish BMI and were checked for cardiovascular diseases. The cardiological exam identified hypertensive patients for subgroup analysis. The initial lab test and treatment were done in the second half of 2017 and throughout 2018. As part of the biochemical exams, the following parameters were determined: Alpha2 Macroglobulin (ALPHA2M); Haptoglobin (Hp); Apolipoprotein A1 (APOA1); total bilirubin (TB); GGT (gamma-glutamyl transferase); glutamic pyruvic transaminase (TGP); glutamic oxaloacetic transaminase (TGO); blood glucose (Gluc); total cholesterol (TC); total triglycerides (TG). Three years after the patients had successfully obtained sustained virological response (SVR), we repeated the tests and measurements for weight and height in order to evaluate the change viral clearance had on these patients.

These biochemical markers were determined using an Architect C8000 analyser (ABBOTT Corporation, USA) using closed-system reagents produced by the same company. ALPHA2M, APOA1, and Hp were determined using immunonephelometric methods, TB was determined by a colourimetric method, and liver enzymes (TGO, TGP, GGT) were determined by enzymatic method while TG, TC and fasting blood glucose with the enzymatic-colourimetric method.

All patients gave written consent and were informed of the procedure involved in the study and that they could withdraw at any time once included. The protocol for this study was set up to follow the ethical guidelines in the Declaration of Helsinki and approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

Statistical analysis

The data collected was stored in Microsoft Excel files (Microsoft® Corp., Redmond, WA, USA), and the statistics were calculated in STATA (StataCorp LLC, Texas, USA). We investigated the relationship between T2DM and biochemical markers in previously HCV-infected patients and the changes to the biomarkers after 3 years after obtaining the SVR. The numerical data was reported as mean ± standard deviation and median of the values. The differences between groups were assessed by the Student's t-test for groups with a normal distribution of values and by Wilcoxon signed rank test for groups with non-normal distribution. Regression analysis was carried out using STATA's multiple linear regression analysis with robust standard errors option after confirming the distribution of variables was normal. The graphical representations were performed with STATA.

Results and Discussion

There was a significant difference between Non-TD2M and TD2M groups regarding the levels of total bilirubin

levels, GGT and fasting blood glucose before treatment. After DAA-treatment, we observed a significant increase in haptoglobin, total cholesterol levels and a decrease in liver transaminases levels (TGO, TGP and GGT). We also observed from the median and quartiles that the triglyceride levels increased, and fasting blood glucose decreased after treatment in both Non-TD2M and TD2M groups. However, this increase didn't reach statistical significance (Table I, Figure 1).

Table I

Biochemical parameters before and after DAA-treatment in diabetic and non-diabetic patients

		ALPHA2M (g/L)		Hp (g/L)		APOA1 (g/L)		BT (mg/dL)		GLU (mg/dL)	
		Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Non-TD2M	**Mean**	3.65	3.68	0.78	1.29*	1.44	1.49	0.83	0.77	105.35	101.90*
	Std. dev	0.66	0.54	0.36	0.58	0.37	0.13	0.24	0.32	14.95	18.11
	Median	3.71	3.67	0.69	1.24	1.39	1.48	0.78	0.70	105.50	101.00
	Q1	3.19	3.40	0.52	0.89	1.23	1.44	0.62	0.62	96.50	93.00
	Q3	4.11	3.95	1.21	1.71	1.57	1.56	1.03	0.89	113.00	109.00
TD2m	**Mean**	3.65	3.49	0.91	1.34*	1.48	1.55	0.86	0.85*	218.30	173.00*
	Std. dev	0.72	0.72	0.52	0.64	0.30	0.22	0.93	0.36	111.88	80.80
	Median	3.74	3.38	0.94	1.36	1.46	1.53	0.64	0.85	176.00	137.00
	Q1	3.10	2.91	1.28	0.69	1.28	1.38	0.44	0.61	141.00	110.00
	Q3	4.26	4.21	1.63	1.81	1.63	1.63	0.88	1.18	264.00	259.00
p value		0.908	0.133	0.317	0.513	0.402	0.276	0.011¥	0.217	< 0.001¥	< 0.001¥
		TGO (U/L)		**TGP (U/L)**		**GGT (U/L)**		**TC (mg/dL)**		**TG (mg/dL)**	
		Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Non-TD2M	**Mean**	79.80	41.25*	105.45	37.70*	98.65	52.2*	156.25	181.45*	108.30	120.45
	Std. dev	42.43	51.69	63.92	30.64	83.49	60.43	48.43	36.53	41.44	55.59
	Median	68.00	24.00	85.00	25.50	65.50	27.00	144.00	185.00	109.50	119.50
	Q1	49.00	20.00	61.50	20.00	48.00	15.00	131.00	166.50	69.50	81.50
	Q3	92.00	37.00	131.00	48.00	111.00	51.00	174.50	207.00	133.50	134.50
TD2m	**Mean**	71.53	46.38*	87.50	47.45*	124.60	61.80*	162.70	183.22*	159.60	138.55
	Std. dev	25.90	51.54	40.96	42.76	119.15	68.40	32.99	34.13	168.76	105.47
	Median	72.00	32.00	82.00	27.00	103.00	48.50	159.00	185.00	103.50	108.00
	Q1	54.00	16.50	59.00	23.00	72.00	19.00	141.00	158.00	73.00	101.00
	Q3	88.00	52.00	108.00	83.00	135.50	58.00	187.00	205.50	128.50	143.00
p value		0.900	0.616	0.397	0.203	0.036¥	0.075	0.094	0.784	0.954	0.603

Data are shown as mean, SD for normally distributed variables, median, 25th and 75th percentiles. *$p < 0.05$ is considered statistically significant for the Student's t-test of mean levels before and after antiviral interferon-free treatment. ¥$p < 0.05$ Student's t-test of the mean difference between Non-TD2M and TD2M groups. Initial, levels before interferon-free treatment; Final, levels after 3 years of SVR; Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus; ALPHA2M, Alpha2 Macroglobulin; Hp, Haptoglobin; APOA1, Apolipoprotein A1; TB, total bilirubin; GGT, gamma-glutamyl transferase; TGP, glutamic pyruvic transaminase; TGO, glutamic oxaloacetic transaminase; Gluc, fasting blood glucose; TC, total cholesterol; TG, total triglycerides

Table II

Increase/decrease in biochemical parameters after antiviral interferon-free treatment

		ALPHA2M (g/L)	Hp (g/L)	APOA1 (g/L)	BT (mg/dL)	GLU (mg/dL)	TGO (U/L)	TGP (U/L)	GGT (U/L)	TC (mg/dL)	TG (mg/dL)
Non-TD2M	**Mean**	0.03	0.50	0.05	-0.05	-46.45	-67.75	-38.55	-3.45	25.20	12.15
	Std. dev	0.51	0.57	0.38	0.36	83.11	47.20	49.17	17.31	54.32	49.82
	Median	0.06	0.31	0.07	-0.13	-36.50	-52.50	-33.00	-2.00	32.50	3.50
	Q1	-0.16	0.10	-0.21	-0.26	-58.50	-87.00	-65.00	-11.00	0.00	-5.50
	Q3	0.33	1.00	0.24	0.23	-7.50	-37.50	-25.50	9.00	53.00	42.50
TD2M	**Mean**	-0.13	0.42	0.07	0.01	-62.80	-40.05	-25.15	-45.30	20.52	-21.05
	Std. dev	0.64	0.48	0.32	0.82	79.46	51.06	51.49	98.81	38.16	137.88
	Median	-0.19	0.40	-0.01	0.13	-56.00	-43.00	-26.00	-27.00	13.50	23.50
	Q1	-0.47	0.13	-0.12	-0.12	-92.00	-58.50	-58.00	-106.00	-2.00	-15.00
	Q3	0.35	0.63	0.36	0.41	-22.00	-21.00	-15.00	7.00	36.00	37.00
p value		0.212	0.459	0.779	0.697	0.371	0.014¥	0.238	0.010¥	0.657	0.156

Data are shown as mean, SD for normally distributed variables, median, 25th and 75th percentiles. ¥$p < 0.05$ Student's t-test of the mean difference between Non-TD2M and TD2M groups. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus; ALPHA2M, Alpha2 Macroglobulin; Hp, Haptoglobin; APOA1, Apolipoprotein A1; TB, total bilirubin; GGT, gamma-glutamyl transferase; TGP, glutamic pyruvic transaminase; TGO, glutamic oxaloacetic transaminase; Gluc, glucose; TC, total cholesterol; TG, total triglycerides

In order to better observe the differences in biochemical parameters after DAA treatment, we summarized the mean and median increase/decrease in Table II. The TGP levels decreased significantly less in diabetic patients compared with non-diabetics. The fasting blood glucose levels decreased slightly in non-diabetics (3.45 mg/dL) and markedly in diabetics (45.30 mg/dL). Nevertheless, we observed a huge standard deviation in fasting blood glucose decrease in TD2M patients, meaning the treatment had very heterogeneous effects in improving the glycaemic control in this group. The decrease was higher in diabetic patients with relatively low glucose levels (first quartile).

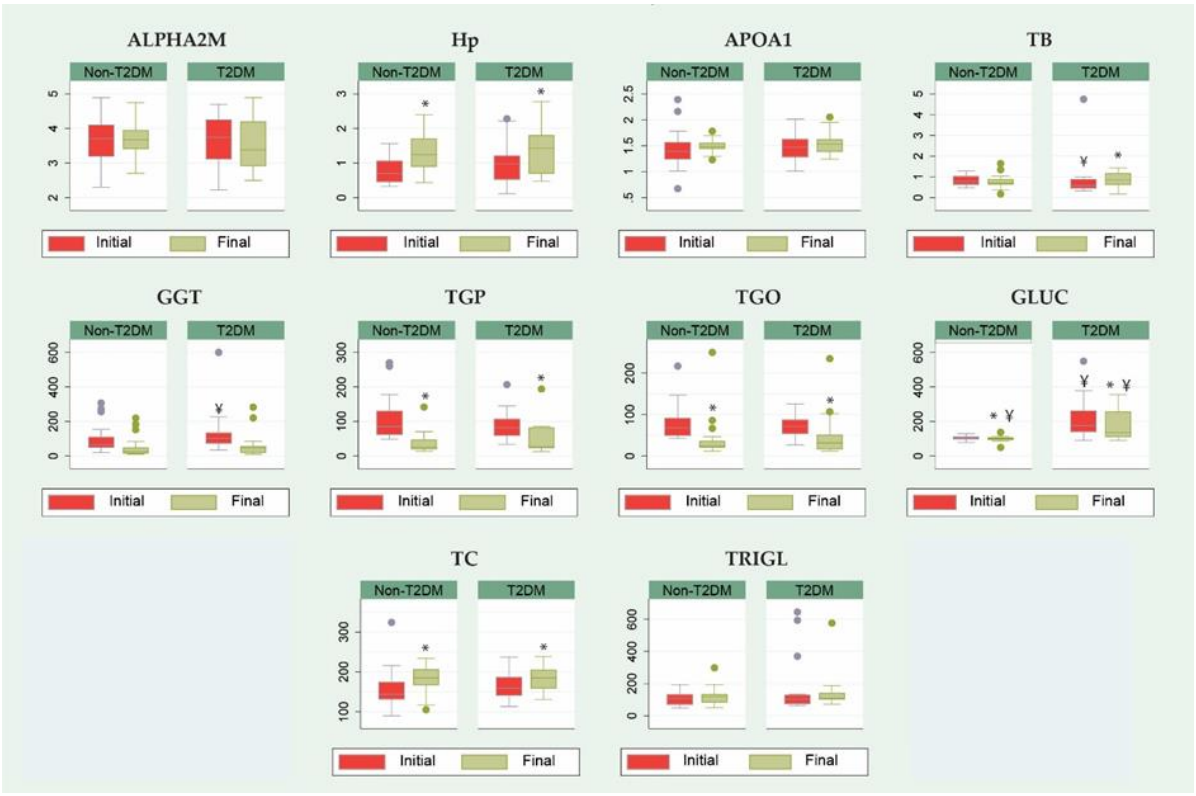

Figure 1.

Biochemical parameters after DAA treatment in diabetic and non-diabetic patients

*p < 0.05 is considered statistically significant for the Student's t-test of mean levels before and after antiviral interferon-free treatment. ¥p < 0.05 Student's t-test of the mean difference between Non-TD2M and TD2M groups. Initial, levels before interferon-free treatment; Final, levels after 3 years of SVR

Correlation between bilirubin and haptoglobin levels and blood glucose levels in diabetic and non-diabetic patients

The multiple regression analysis revealed that total bilirubin levels are a protective factor for blood glucose increase following DAA treatment in diabetic patients, 1 mg/dL increase in BT leading to a decrease by 34 mg/dL of blood glucose (Table III).

Table III

Regression analysis of blood glucose values after DAA treatment, dependent on total bilirubin levels, adjusting for initial liver transaminases values

	Non-TD2M			TD2M		
Parameter	**Regression coefficient ± SD**	**t value**	**p value**	**Regression coefficient ± SD**	**t value**	**p value**
Blood glucose	0.805 ± 0.17 mg/dL	4.73	0.00	0.600 ± 0.13 mg/dL	4.62	0.00
TGO	-0.252 ± 0.08 U/L	-3.35	0.002*	-0.413 ± 0.61 U/L	-0.67	0.504
TGP	0.049 ± 0.05 U/L	0.90	0.372	0.384 ± 0.32 U/L	1.19	0.244
GGT	0.059 ± 0.04 vU/L	1.64	0.111	-0.140 ± 0.12 U/L	-1.20	0.238
TB	2.388 ± 11.54 mg/dL	0.21	0.837	-34.786 ± 15.33 mg/dL*	-2.27	0.030*
Constant term	24.175 ± 21.36 mg/dL	1.13	0.266	85.00 ± 36.80 mg/dL	2.31	0.027

Data are shown as mean ± SD for normally distributed variables, and *p < 0.05 for Student's t-test of mean difference in normotensives compared with hypertensive is considered statistically significant. *p < 0.05 – significant statistical regression coefficient. All statistical models had significant predictive capability. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, type 2 Diabetes Mellitus; TB, total bilirubin; GGT, gamma-glutamyl transferase; TGP, glutamic pyruvic transaminase; TGO, glutamic oxaloacetic transaminase

If we further add haptoglobin to the model, we observe that in non-TD2M patients, the haptoglobin has led to a decrease in fasting blood glucose levels with 14 mg/dL for 1 mg/dL increase in haptoglobin levels. In the case of T2DM patients, we observe a significant decrease in fasting blood glucose levels with 29 mg/dL for 1 mg/dL increase in haptoglobin levels and respectively a decrease with 43 mg/dL for 1 mg/dL increase in total bilirubin levels.

Table IV

Regression analysis of blood glucose values after interferon-free antiviral treatment, dependent on total bilirubin levels and haptoglobin levels, adjusting for initial liver transaminases values

	Non-TD2M			TD2M		
Parameter	**Regression coefficient ±SD**	**t value**	**P value**	**Regression coefficient ±SD**	**t value**	**p value**
Blood glucose	0.818 ± 0.15 Mg/dL	5.39	0.00	0.631 ± 0.13 mg/dL	4.94	0.00*
TGO	-0.343 ± 0.94 U/L	-3.66	0.001	-0.635 ± 0.70 g/L	-0.90	0.372
TGP	0,.913 ± 0.04 U/L	2.15	0.039	0.377 ± 0.342 U/L	1.1	0.278
GGT	0.056 ± 7.96 U/L	1.91	0.065	-0.072 ± 0.12 U/L	-0.58	0.567
TB	10.503 ± 6.59 mg/dL	1.32	0.196	-43.170 ± 14.71 mg/dL*	-2.93	0.006
Hp	-14.420 ± 6.59* mg/dL	-2.19	0.036	-29.438 ± 22.24* mg/dL	-1.32	0.195
Constant term	3.449 ± 15.49 mg/dL	1.97	0.058	0.54 ± 0.30 mg/dL	3.13	0.004

Data are shown as mean ± SD for normally distributed variables, and *p < 0.05 for Student's t-test of mean difference in normotensives compared with hypertensive is considered statistically significant. *p < 0.05 – significant statistical coefficient. All statistical models had significant predictive capability. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, type 2 Diabetes Mellitus; TB, total bilirubin; GGT, gamma-glutamyl transferase; TGP, glutamic pyruvic transaminase; TGO, glutamic oxaloacetic transaminase; Hp, haptoglobin

Correlation between bilirubin and haptoglobin levels and blood glucose levels in diabetic and non-diabetic patients

The haptoglobin values before treatment were significantly lower in hypertensive patients (0.80 g/L) compared with normotensives (1.04 g/L) but not in Non-TD2M patients. The differences were maintained after DAA treatment. Also, the haptoglobin levels increased significantly after the treatment, excepting the hypertensive patients without diabetes (Table V).

Table V

Differences in biomarkers' levels (haptoglobin, cholesterol, bilirubin) in hypertensive *vs.* normotensive patients in diabetics and non-diabetics

Haptoglobin levels						
	Non-TD2M		**p value**	**TD2M**		**p value**
	Without HT	**With HT**		**Without HT**	**With HT**	
Before treatment	0.77 ± 0.06 g/L	0.82 ± 0.05 g/L	0.695	1.04 ± 0.06 g/L	0.80 ± 0.05 g/L	0.023*
After treatment	1.26 ± 0.09 g/L	1.37 ± 0.07 g/L	0.662	1.33 ± 0.04 g/L	1.49 ± 0.05g/L	0.045*
Increase after treatment	0.49 ± 0.11 g/L¥	0.54 ± 0.30 g/L	0.886	0.33 ± 0.16 g/L¥	0.64 ± 0.24 g/L¥	0.047*
Cholesterol levels						
	Non-TD2M		**p value**	**TD2M**		**p value**
	Without HT	**With HT**		**Without HT**	**With HT**	
Before treatment	162.75 ± 8.92 mg/dL	130.25 ± 10.16 mg/dL	0.090	166.38 ± 6.09 mg/dL	153.00 ± 9.93 mg/dL	0.257
After treatment	189.06 ± 6.15 mg/dL	151 ± 9.65 mg/dL	0.007*	185.07 ± 6.46 mg/dL	178.36 ± 10.09 mg/dL	0.585
Increase after treatment	26.31 ± 10.83 mg/dL¥	20.75 ± 14.02 mg/dL	0.844	18.69 ± 8.88 mg/dL¥	25.36 ± 14.16 mg/dL	0.627
Total bilirubin						
	Non-TD2M		**p value**	**TD2M**		**p value**
	Without HT	**With HT**		**Without HT**	**With HT**	
Before treatment	0.80 ± 0.041 mg/dL	0.90 ± 0.098 mg/dL	0.307	0.95 ± 0.200 mg/dL	0.57 ± 0.054 mg/dL	0.254
After treatment	0.76 ± 0.053 mg/dL	0.84 ± 0.131 mg/dL	0.509	0.91 ± 0.071 mg/dL	0.68 ± 0.076 mg/dL	0.046*
Increase after treatment	-0.05 ± 0.058 mg/dL	-0.03 ± 1.728 mg/dL	0.920	-0.03 ± 0.176 mg/dL	0.11 ± 0.0734 mg/dL	0.626

Data are shown as mean ± SD for normally distributed variables, and *p < 0.05 for Student's t-test of mean difference in normotensives compared with hypertensive is considered statistically significant. ¥p < 0.05 for Student's t-test of mean difference before and after treatment is considered statistically significant. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus; without HT, without hypertension; with HT, with hypertension

Our tests also show a significant increase in TC after treatment only in the case of non-T2DM patients with hypertension. The total bilirubin values after treatment were significantly higher in hypertensive patients with T2DM (0.11 mg/dL increase).

We defined high total bilirubin as values of BT over 0.8 mg/dL. We observed that in patients with high BT, the hypertension prevalence was significantly lower in diabetic patients (11.76% *vs*. 39.13%) but not in non-diabetic patients (Table VI).

We observe from Table VII that there is a strong correlation in the association of Hp and TC in the case of T2DM patients and the presence of hypertension (0.029), and in the case of non-T2DM patients without hypertension, an even stronger connection (0.014).

We defined overweight as a BMI value of 25 and above. Our study shows that there is a direct correlation between haptoglobin levels and patients' BMI in the case of diabetic patients (0.018).

Table VI

The effect of high bilirubin levels on the prevalence of hypertension in diabetic *vs*. non-diabetic patients

	Non-TD2M				TD2M		
	High BT (n = 18)	**Normal BT (n = 22)**	**p value**		**High BT (n = 17)**	**Normal BT (n = 23)**	**p value**
Hypertensive patients (n = 8)	4	4	0.751	**Hypertensive patients (n = 11)**	2	9	0.045*
Normotensive patients (n = 32)	14	18		**Normotensive patients (n = 29)**	15	14	
HT prevalence	22.22%	18.18%		**HT prevalence**	11.76%	39.13%	

Data are shown as the number of cases and percentage and *p < 0.05 for the Chi-squared test of the significant effect of high BT on hypertension prevalence. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, type 2 Diabetes Mellitus; HT, Hypertension

Table VII

Comparative regression of haptoglobin levels with total cholesterol levels according to the status of hypertension

	Non-TD2M				TD2M			
	Without HT	**With HT**	**p value without HT**	**p value with HT**	**Without HT**	**With HT**	**p value without HT**	**p value with HT**
TC	0.003 ± 0.001 g/L	0.82 ± 0.05 g/L	0.014*	0.141	0.005 ± 0.004 g/L	0.006 ± 0.002 g/L	0.206	0.029*
Constant term	1.26 ± 0.09 g/L	1.37 ± 0.07 g/L	0.178	0.021	0.2411 ± 0.600 g/L	-0.133 ± 0.367 g/L	0.691	0.726

Data are shown as mean ± SD and *p < 0.05 for the Student's t-test of mean difference in normotensives compared with hypertensive is considered statistically significant. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus; without HT, without hypertension; with HT, with Hypertension, TC, total cholesterol

Table VIII

The relationship of haptoglobin levels with total cholesterol, triglycerides levels and overweight status

	Non-TD2M			TD2M		
	Regression coefficient ± SD	**t value**	**p value**	**Regression coefficient ± SD**	**t value**	**p value**
TC	0.002 ± 0.001 mg/dL	1.56	0.128	0.005 ± 0.003 mg/dL	1.72	0.094
TG	-0.002 ± 0.001 mg/dL	-1.69	0.099	-0.0005 ± 0.0006 mg/dL	0.86	0.397
Overweight	0.120 ± 0.12	1.01	0.319	0.429 ± 0.173	2.47	0.018*
Constant term	0.677 ± 0.22 g/L	3.09	0.004	0.247 ± 0.479 g/L	-0.52	0.609

Data are shown as mean± SD and *p < 0.05 for the Student's t-test of mean difference in normotensives compared with hypertensive is considered statistically significant. Non-T2DM, non-type 2 Diabetes Mellitus; T2DM, Type 2 Diabetes Mellitus; without HT, without hypertension; with HT, with Hypertension, TC, total cholesterol; TG, Total triglycerides; Overweight, BMI > 25. All statistical models had significant predictive capability

The relationship between bilirubin metabolism and the occurrence, development and prognosis of disease has become a research focus. In recent years studies have been made to understand the relationship between bilirubin concentration and the risk of diabetic complications. Studies have shown that higher serum bilirubin levels could decrease the risk of T2DM, and lower serum bilirubin levels were found in diabetes [11, 12]. Our results are aligned with these studies showing clearly that patients with higher levels of TB, while still in the normal range, had better glycaemic control and drops in fasting blood glucose levels (Table II and Table III).

In a key review from 2016, it was reported that slightly elevated bilirubin levels had a protective effect on cardiovascular disease and diabetes. This was correlated with enhanced oxidative stress that affects bilirubin metabolism and may be considered a possible bio-medical strategy in these diseases. Several trials have shown that a high concentration of bilirubin has a beneficial impact on diabetic complications, although this is not agreed upon by all researchers [13]. Our

results also indicated that higher TB levels are linked to a protective role in preventing hypertension in diabetic patients (Table VI).

Other studies have indicated that high bilirubin levels have a protective effect on diabetic complications [14-17]. Wang *et al*. showed that serum bilirubin levels increased in new-onset diabetes [18], although other studies do not sustain this relationship [19, 20]. The involvement of bilirubin levels in glycaemic control remains controversial. Our study revealed that increased total bilirubin levels were linked to a decrease in blood glucose levels in T2DM patients. One possible explanation could be that oxidative stress increases in the initial stage of T2DM because the body is exposed to a high-glucose environment. As a result, the liver might increase bilirubin production to exert its antioxidant effects. However, the prolonged duration of diabetes and chronic hyperglycaemia results in continuous oxidative stress that increases the consumption of bilirubin. Thus, the serum bilirubin level decreases [21, 22]. It is considered that bilirubin improves insulin sensitivity, acting as an insulin sensitizer by inhibiting the endoplasmic reticulum stress and inflammation and improves glycaemic control in T2DM. Perhaps in this way, it is observed that bilirubin is positively correlated with insulin sensitivity in the case of glucose metabolism abnormalities [23]. Serum bilirubin concentration was shown to have a positive correlation with antioxidative enzymes and a negative correlation with oxidative stress levels. Thus bilirubin may protect against diabetic complications [24].

Insulin resistance is strongly associated with obesity-related impaired glucose regulation (IGR) and T2DM, in which oxidative stress and inflammation are potential etiological factors [25]. Recent studies have shown that a mild increase in bilirubin levels while within the physiological range has a protective effect against obesity-related metabolic disease [26].

Hp is expressed in various tissues and cell types, including white adipose tissue (WAT) [27]. A study has observed that patients with chronic HCV infection had lower levels of Hp compared to control subjects. The same effects were observed in the case of HCV-infected patients without cirrhosis [28]. The results are similar to what we observed in our patients, where a significant increase in Hp levels after SVR was obtained, showing that DAA treatment is an important step to better controlling T2DM (Table I and Table II). The Hp2 allele is mainly related to metabolic disease, and studies have positively correlated Hp2 with diabetic complications, being used as a biomarker for obesity [29]. In addition, when the body is in a stressed state, such as inflammation, trauma, infection, or other pathological conditions, blood Hp levels increase significantly [30].

Obesity cohort studies have confirmed that circulating Hp can be used as a biomarker in identifying simple obesity [31]. A study on mice showed that in the case of Hp deficiency, obesity-associated insulin resistance and hepatosteatosis are diminished [32]. It is now believed that adipose tissue is an endocrine organ, and white adipose tissue can secrete Hp. Obese patients present chronic inflammation and insulin resistance, two factors that interact with each other. Hp is an important participant in triggering WAT inflammation during the onset of obesity and is expressed in adipocytes [29]. In our results, it is evident that Hp levels are strongly correlated with obesity and obesity-related diseases, such as higher cholesterol, but no correlation was observed in regards to triglycerides (Table VIII). Currently, studies show evidence that Hp is closely related to various complications of diabetes. One such study indicated that urine Hp might be a new biomarker for the risk of death in patients with T2DM [33]. We also observed a strong correlation of Hp levels with total cholesterol for T2DM patients with Hypertension (Table VII). A recent study demonstrated that intensive glucose-lowering therapy was an effective way to prevent cardiovascular disease events in the case of T2DM patients with the Hp2-2 genotype [34].

An investigation on Hp polymorphism and serum Hp concentration reported a comparison of LDL cholesterol, HDL cholesterol and triglycerides levels of overweight subjects ($25 \leq BMI < 30$) and normal-weight subjects ($BMI < 25$) [35].

In our study, Hp level was positively correlated with HDL cholesterol and total cholesterol level but not with triglyceride level through linear regression analyses (Table VII).

Conclusions

Our results have shown that the elimination of HCV infection is beneficial not only in preventing liver complications like cirrhosis and HCC, but also in better controlling T2DM and its complications. HCV clearance improves not only the biomarkers of liver function, but also TB and Hp, which are strongly connected with insulin resistance in T2DM, leading to better control of blood glucose levels, obesity, and lipid metabolism. These two biomarkers should be further investigated not only to understand better the physiopathological pathways by which they affect TD2M progression but also how their levels might be raised in the pursuit of better control of diabetes, cardiovascular complications, and other diseases.

Conflict of interest

The authors declare no conflict of interest.

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