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Insulin Resistance and Anti-HCV Therapy Outcomes in Chronic HCV Patients in Najran, Saudi Arabia.

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ABSTRACT

The association between HCV infection, insulin resistance and the treatment outcomes has been documented all over the world. We aimed to investigate HOMA-IR in either Responders or non-Responders to therapy with pegylated interferon and ribavirin in HCV patients. HCV patients (175 men) were divided into three groups; the first group included all treated HCV patients (175); the second group, Responders (107) to therapy and had undetectable PCR levels for HCV-RNA after 24 weeks of treatment; and the third group Non-Responders (68) included HCV patients who did not respond to anti-HCV therapy and had detectable HCV-RNA levels after 24 weeks of treatment. Most of HCV patients respond to the treatment with pegylated interferon and ribavirin (61.14%) with undetectable serum HCV-RNA. The non-responders were 38.86% had a mean serum HCV-RNA of 2401889± 1021016 IU/ml. The mean HOMA-IR was 2.61±1.23 for the Responders and 21.92±11.31 for the Non-Responders (P<0.0001). There was a significant increase in the mean serum glucose and insulin levels and BMI in Non-Responders than the Responders. We conclude that HOMA-IR index represents a prognostic value for antiviral therapy regardless of their HCV genotypes. Chronic HCV patients who are candidates for antiviral therapy should be checked for insulin resistance before treatment.

Keywords: HCV, Insulin resistance, HOMA-IR, RNA, Najran.

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INTRODUCTION

Hepatitis C virus (HCV) infection is considered a major global health problem with a high rate of chronic evolution. Chronic HCV infects more than 170 million individuals worldwide[1,2]. Hepatitis C virus can successfully escape from immune system, which results in persistence of infection in about 80% of the patients. The deleterious consequences are cirrhosis and hepatocellular carcinoma. HCV is considered the most frequent cause for hepatocellular carcinoma (HCC) [4-7]. HCV infection is the most serious blood borne infection in the Middle East and constitutes a major health problem in golf area. In Saudi Arabia, viral hepatitis ranked the second most common reportable viral disease in 2007, with almost 9000 new cases diagnosed in that year[8]. Mansour et al., 2011, reported a prevalence of 1.1% for anti-HCV antibodies among 500 newly married Saudi couples [9].

Insulin resistance (IR) (a precursor to diabetes) is a chronic metabolic syndrome characterized by a lot of abnormalities including altered glucose tolerance [10,11]. HCV infection is an important risk factor for IR [12]. IR is commonly associated with chronic liver disease, and the interaction between HCV infection and IR is a major public health issue [13]. Interestingly, co-existence of IR and chronic HCV is a common event ranging from 30% to 70% of patients with chronic HCV [14].

HCV infection and IR have a potential synergism on the severity of liver disease, so a better understanding of the clinical pathogenesis of the relationship between them should be focused [15]. Moreover, Mohamed et. al., 2011 added that IR occurs early in HCV infection and may synergize with viral hepatitis in HCC development [13]. Because of their potential synergism on liver disease severity, a better understanding of their pathogenesis is needed[15-18]. Also, it was postulated that viral IR may not reduce the rate of response to anti-HCV therapy to the same extent that metabolic IR does [19]. Moreover, Khattab et al., 2010 reported that HOMA-IR has a role as a predictive factor of therapy-induced HCV virus eradication [20].

Insulin resistance is also a predictor of poorer treatment response in patients with HCV [1, 21-24]. Moreover, clearance of HCV with antiviral therapy could improve insulin sensitivity [18,25,26]. Insulin resistance has been found to be an independent factor predicting sustained response to peginterferon plus ribavirin in patients with chronic HCV [27-29].

HCV interacts with lipid metabolism leading to steatosis, causing wide adipocytokines changes and impairs glucose metabolism leading to increased prevalence of IR and type 2 diabetes [20,30,31]. The association between Chronic HCV infection with IR and the response to anti-HCV therapy outcomes has been documented in different parts of the world; nevertheless, no conclusive data is available in Saudi Arabia. In the present study, we aimed to investigate and compare insulin resistance (HOMA-IR) in Chronic HCV patients who were either Responders or non-Responders to anti-HCV therapy with pegylated interferon and ribavirin.

MATERIALS AND METHODS

This study was a prospective, randomized, open-label trial. Blood samples were collected from Chronic HCV Saudi patients in Najran area, KSA. The patients were referred to the Najran Regional Laboratory from Hepatology Units of all hospitals in Najran area, particularly King Khalid and Najran General Hospitals. Between January 2012 and March 2014, 175 male patients with chronic hepatitis C virus (HCV) aged 28-62 years (median 44 years) were consecutively enrolled. Subjects co-infected with hepatitis B virus, HIV or any other causes of liver disease were excluded.

Study Protocol

Subjects were divided into three groups; the first group included all HCV patients (175 male individuals) who received standard anti-HCV therapy with pegylated interferon and ribavirin for 6-12 months according to HCV genotype; the second group, Responders (107 male individuals) included HCV patients who respond to anti-HCV therapy and had undetectable PCR levels for HCV-RNA after 24 weeks of treatment; and the third group (Non-Responders, 68 male individuals) included HCV patients who did not respond to anti-HCV therapy and had detectable PCR levels for HCV-RNA after 24 weeks of treatment. Subjects underwent a medical interview, physical examination including anthropometric measurements, fasting laboratory evaluation, and liver biopsy before the beginning of treatment. Patients received standard anti-HCV therapy

with pegIFN-α-2a 180μg subcutaneously weekly plus weight-based RBV (Pegasys® and Copegus®, Roche Laboratory, Nutley, NJ USA), regardless of HCV genotype (800 mg/day for <65 kg; 1000 mg/day for ≥ 65 kg and ≥ 85 kg; 1200 mg/day for > 85 kg). All patients were contacted by telephone and invited to join the study. This study was approved by the Ethics of Najran community and all subjects were informed about the aim of the study.

Virological Assessment

Quantification of serum HCV-RNA was performed using COBAS TaqMan 48 (Roche Diagnostics, a division of F. Hoffmann-La Roche, 4070 Basel, Switzerland) which is a bench top analyzer for quantitative and qualitative nucleic acid testing using PCR technology with homogenous amplification and real-time fluorescence multicolor detection. The detection limit was 600 IU/ml.

Histological Assessment

All patients underwent baseline liver biopsy before treatment to assure that they are suitable candidates for anti-HCV therapy with pegylated interferon and ribavirin. Liver biopsies were evaluated by experienced pathologists who were unaware of the virological status of patients. The liver biopsy histologic lesions were analyzed according to Ishak et al., 1995 [32] classification system.

BMI and IR Calculations

BMI was calculated by the body weight in kilograms divided by the square of the height in meters. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) is an indirect marker of insulin resistance. HOMA-IR was calculated according to Matthews et al., 1985 [33] using the Formula HOMA-IR = [Fasting insulin (μU/ml)⁻ fasting glucose (mmol/L)/22.5].

Blood samples

Study participants were assessed in the morning after an overnight fasting (12 hours). Blood samples were collected from 175 male individuals. Of these, 107 (61.14%) HCV patients were responders to anti-HCV therapy with pegylated interferon and ribavirin, and 68 (38.86%) HCV patients were non-Responders. Serum was collected and kept under -70˚C until use.

Biochemical measurements

Insulin was estimated by Access 2 machine (Beckman Coulter, Inc.) by using direct chemiluminescent technology. Glucose and liver functions including gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TB), albumin (ALB), total protein (TP) were measured using using COBAS INTEGRA automatic analyzers (Roche Diagnostics GmbH, USA).

Statistical analysis

Statistical analyses was performed using Graph Pad InStat 3 (GraphPad Software, Inc. 2236 Avenida de la PlayaLa Jolla, CA 92037 USA). Analysis of variance (ANOVA) was used to analyze the differences between group means. We used a two-tailed test, and a value of $P<0.05$ was considered statistically significant.

RESULTS

In the present study blood samples were collected from Chronic HCV Saudi men in Najran area, KSA. It included 175 male patients with chronic hepatitis C virus (HCV) aged 28-62 years (median 44 years) were consecutively enrolled. Subjects were divided into three groups; the first group (All HCV patients) included all HCV patients who received standard anti-HCV therapy with pegylated interferon and ribavirin (PEG-IFN + RBV) for 6-12 months according to HCV genotype; the second group (Responders) included HCV patients who respond to anti-HCV therapy and had undetectable PCR levels for HCV-RNA; and the third group (Non-Responders) included HCV patients who did not respond to anti-HCV therapy and had detectable PCR levels for HCV-RNA.

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The baseline characteristics of the patients included in the present study are summarized in table (1). The mean age was 39.37± 15.58 years for the total HCV patients group, 35.7 ± 19.15 years for the Responders patients to therapy and 39.37± 20.58 years for the Non-Responders patients to therapy. There was a non-significant difference in mean age among the studied groups. The mean height was 1.70 ± 0.17 m for the total HCV patients group, 1.71 ± 0.09 m for the Responders patients to therapy and 1.65 ± 0.11 m for the Non-Responders patients to therapy. There was a significant difference in mean height among the studied groups (P=0.01). The mean weight was 81.89 ± 16.29 kg for the total HCV patients group, 69.45 ± 8.14 kg for the Responders patients to therapy and 77.15 ± 10.76 kg for the Non-Responders patients to therapy. There was an extremely significant difference in mean weight among the studied groups (P<0.0001). The mean body mass index (BMI) was 30.59 ± 8.98 kg/m2 for the total HCV patients group, 25.83 ± 9.21 kg/m2 for the Responders patients to therapy and 35.63 ± 5.13 kg/m2 for the Non-Responders patients to therapy. There was an extremely significant difference in mean BMI among the studied groups (P<0.0001). The mean serum glucose was 117.35 ± 55.34 mg/dl for the total HCV patients group, 109.3 ± 50.53 mg/dl for the Responders patients to therapy and 150.13 ± 61.63 mg/dl for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum glucose among the studied groups (P<0.0001). The mean serum insulin level was 35.22 ± 30.62 µU/ml for the total HCV patients group, 13.95 ± 7.89 µU/ml for the Responders patients to therapy and 57.08 ± 32.1 µU/ml for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum insulin among the studied groups (P<0.0001). The mean serum PCR-RNA (IU/ml) before treatment was 3152673± 1368516 IU/ml for the total HCV patients group, 2851485 ± 1248763 IU/ml for the Responders patients to therapy and 3005875 ± 1685327 IU/ml for the Non-Responders patients to therapy. There was a non-significant difference in mean age among the studied groups (P=0.21).

Table 1: Baseline characteristics of patients included in the present study

Item	Total HCV patients (n=175)	Responders to therapy (n=107)	Non-Responders to therapy (n=68)	P value
Age (years)	39.37± 15.58	35.7 ± 19.15	39.37± 20.58	0.12
Height (m)	1.70 ± 0.17	1.71 ± 0.09	1.65 ± 0.11	0.01*
Weight (kg)	81.89 ± 16.29	69.45 ± 8.14	77.15 ± 10.76	<0.0001***
BMI (kg/m2)	30.59 ± 8.98	25.83 ± 9.21	35.63 ± 5.13	<0.0001***
Glucose (mg/dl)	117.35 ± 55.34	109.3 ± 50.53	150.13 ± 61.63	<0.0001***
Insulin (µU/ml)	35.22 ± 30.62	13.95 ± 7.89	57.08 ± 32.1	<0.0001***
PCR-RNA (IU/ml) before treatment	3152673± 1368516	2851485 ± 1248763	3005875 ± 1685327	0.21

Table (2) demonstrates the metabolic, biochemical and virological evaluation of patients included in the present study after treatment. All HCV patients received standard anti-HCV therapy with pegylated interferon and ribavirin for 6-12 months according to HCV genotype. The mean serum PCR-RNA (IU/ml) after treatment was non detected for the Responders patients to therapy and was 2401889 ± 1021016 IU/ml for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum PCR-RNA between the responder and non- responder groups (P<0.0001). The mean Insulin Resistance (HOMA-IR) after treatment was 14.24 ± 19.61 for the total HCV patients group, 2.61 ±1.23 for the Responders patients to therapy and 21.92±11.31 for the Non-Responders patients to therapy. There was an extremely significant difference in mean Insulin Resistance among the studied groups (P<0.0001).

The mean serum glucose after treatment was 125.42 ± 48.28 mg/dl for the total HCV patients group, 112.85± 43.25 mg/dl for the Responders patients to therapy and 165.27± 44.15 mg/dl for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum glucose among the studied groups (P<0.0001). The mean serum insulin after treatment was 33.73± 21.91 (µU/ml) for the total HCV patients group, 12.63± 6.27 (µU/ml) for the Responders patients to therapy and 53.51±29.71 (µU/ml) for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum insulin among the studied groups (P<0.0001).

The mean serum BMI after treatment was 30.37± 13.55 kg/m2 for the total HCV patients group, 23.27± 8.46 kg/m2 for the Responders patients to therapy and 33.38±7.94 kg/m2 for the Non-Responders

patients to therapy. There was an extremely significant difference in mean BMI among the studied groups (P<0.0001).

Table 2: Metabolic, biochemical and virological evaluation of patients included in the present study after treatment standard anti-HCV therapy with pegylated interferon and ribavirin

Item	Total HCV patients 175 (100%)	HCV Responders 107 (61.14%)	HCV Non-Responders 68 (38.86%)	P value
PCR-RNA (IU/ml) after treatment		Not detected	2401889 ± 1021016	<0.0001***
Insulin resistance (HOMA-IR)	14.24 ± 19.61	2.61 ±1.23	21.92±11.31	<0.0001***
Glucose (mg/dl) after treatment	125.42 ± 48.28	112.85± 43.25	165.27± 44.15	<0.0001***
Insulin (µU/ml) after treatment	33.73± 21.91	12.63± 6.27	53.51±29.71	<0.0001***
BMI (kg/m2) after treatment	30.37± 13.55	23.27± 8.46	33.38±7.94	<0.0001***
GGT (U/L)	48.16± 24.87	40.46±16.27	55.36±21.32	<0.0001***
ALT (U/L)	71.98± 42.61	54.54±25.60	86.96±39.56	<0.0001***
AST (U/L)	68.75± 50.33	51.11±32.45	80.96±40.99	<0.0001***
ALP (U/L)	94.77± 42.41	72.03±33.79	99.59±39.9	<0.0001***
Albumin (g/L)	40.32± 5.33	42.02±5.81	38.81±4.68	0.0005***
Total protein (g/L)	72.68± 7.15	73.22±6.84	71.4±7.72	0.26
Total Bilirubin (mmol/L)	13.45± 7.36	12.47± 7.18	15.40± 8.09	0.04*

The mean serum gamma glutamyl transferase (GGT) levels after treatment was 48.16± 24.87 U/L for the total HCV patients group, 40.46±16.27 U/L for the Responders patients to therapy and 55.36±21.32 U/L for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum GGT levels among the studied groups (P<0.0001).

The mean serum alanine aminotransferase (ALT) levels after treatment was 71.98± 42.61 U/L for the total HCV patients group, 54.54±25.60 U/L for the Responders patients to therapy and 86.96±39.56 U/L for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum ALT levels among the studied groups (P<0.0001).

The mean serum aspartate aminotransferase (AST) levels after treatment was 68.75± 50.33 U/L for the total HCV patients group, 51.11±32.45 U/L for the Responders patients to therapy and 80.96±40.99 U/L for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum AST levels among the studied groups (P<0.0001).

The mean serum alkaline phosphatase (ALP) levels after treatment was 94.77± 42.41 U/L for the total HCV patients group, 72.03±33.79 U/L for the Responders patients to therapy and 99.59±39.9 U/L for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum ALP levels among the studied groups (P<0.0001).

The mean serum Albumin levels after treatment was 40.32± 5.33 g/L for the total HCV patients group, 42.02±5.81 g/L for the Responders patients to therapy and 38.81±4.68 g/L for the Non-Responders patients to therapy. There was an extremely significant difference in mean serum Albumin levels among the studied groups (P=0.0005).

The mean serum total protein levels after treatment was 72.68± 7.15 g/L for the total HCV patients group, 73.22±6.84 g/L for the Responders patients to therapy and 71.4±7.72 g/L for the Non-Responders patients to therapy. There was a non significant difference in mean serum total protein levels among the studied groups (P=0.26).

The mean serum total bilirubin levels after treatment was 13.45± 7.36 mmol/L for the total HCV patients group, 12.47± 7.18 mmol/L for the Responders patients to therapy and 15.40± 8.09 mmol/L for the

Non-Responders patients to therapy. There was a significant difference in mean serum total bilirubin levels among the studied groups (P=0.04).

DISCUSSION

The present study demonstrated that most of chronic HCV patients respond to the treatment with PEG-IFN + RBV, where 61.14% (107/175) of patients were responders to the anti-viral treatment. The responders had undetectable serum HCV-RNA by quantitative PCR after the end of treatment. The non-responders were 38.86% (68/175) patients did not respond to the anti-viral treatment with PEG-IFN + RBV. Their mean serum HCV-RNA was 2401889± 1021016 IU/ml at the end of treatment by PCR.

The association between Chronic HCV infection with the IR and the treatment outcomes has been documented in different parts of the world, (21, 27, 22, 1 and 15); nevertheless, no conclusive data is available in Saudi Arabia. Our results indicate that HOMA-IR has a great effect on the response to anti-HCV treatment. The mean HOMA-IR after treatment was 2.61±1.23 for the Responders and 21.92±11.31 for the Non-Responders patients to therapy (P<0.0001). At the same time, there was an extremely significant increase in the mean serum glucose and insulin levels and BMI in the Non-Responders patients compared with that of the Responders patients to therapy. This was accompanied with a significant improvement in the liver function parameters of the Responders compared with that of the Non-Responders patients to therapy, table (2).

A variety of viral, environmental and host genetic factors were found to be contributed to the clinical outcomes of patients infected with HCV and influence response to interferon therapy1. Chronic HCV infection has been linked to IR and T2DM and their incidence is much more prevalent in HCV patients than in healthy controls [28,34]. Many reports provide evidence that HCV infection significantly increased the incidence of glucose abnormalities in chronically infected patients[10,22, 23]. The prevalence of T2DM in chronic HCV patients ranges from 24-50% and this frequency is about 5 times greater than the rest of hepatic cirrhosis [35,36]. Mangia et al., 1998 reported that the occurrence of T2DM in patients with HCV infection was a consequence of impaired glucose metabolism related to cirrhosis [37]. On the other hand, many reports provide evidence that eradication of HCV infection significantly reduces the incidence of glucose abnormalities in chronic HCV patients [10,34]. In addition, IR was found to promote the occurrence of hepatic steatosis in chronic HCV patients. It was previously reported that, IR is probably the "first step" in non-alcoholic steato-hepatitis and its incidence is about 2.5 fold more frequent in HCV patients as compared to the general population [27,28]. Moreover, the improvement of the sustained virological response (SVR) during HCV treatment was found to reduce both IR and hepatic steatosis[38]. Hepatic steatosis is increasingly recognized as the hepatic manifestation of metabolic syndrome and is an important cause of liver-related morbidity and mortality [23].

Metabolic factors, including IR, were reported to influence not only the natural course of HCV infection and to a lower sustained viral response rate, as well as associate to an accelerated hepatic fibrosis progression, to a worse prognosis when hepatic cirrhosis is present and increased risk of hepatocellular carcinoma [28]. In addition, a higher prevalence of IR and diabetes mellitus in chronic HCV resulted in more than 33% fatty cells on liver biopsy and are responsible for decreased rate of SVR [21,22].

Our results agree with that reported by many authors. Bortoletto et al., 2010, found that IR reduces response to pegylated-interferon (PEG-IFN)/ribavirin in chronic HCV patients. Younossi and McCullough, 2009, reported that metabolic abnormalities have been shown to influence response to chronic HCV treatment such that the presence of IR or obesity reduces the likelihood of SVR [23]. In addition, hyperinsulinaemia was found to reduce the cellular response to Pegylated-interferon in chronic HCV patients with IR [39]. At the time that HCV infection appears to exacerbate the metabolic syndrome by eliciting IR [23], conversely, a good response to anti-HCV treatment and SVR has been demonstrated to ameliorate IR and improve beta-cell function. Moreover, it was postulated that patients with chronic HCV Who are candidates to antiviral therapy with PEG-IFN + RBV must be checked for IR before being started on treatment since HOMA-IR index presents a prognostic value for SVR in these patients, regardless of their HCV genotypes [40,41].

On the other hand, in patients who achieve viral eradication, IR and hepatic steatosis may regress, and return if viral infection recurs, which once again indicates an intrinsic steatosis and IR promoter action by

HCV [28]. Increased HOMA-IR values, regardless in diabetic or non-diabetic, was found to be correlated with the development of HCC in chronic HCV patients [13,42].

The pathophysiology of the mutual relationship between HCV and IR is not fully understood. But, it could be explained by results indicating that HCV proteins Core, NS-3 and NS-5 are mainly involved in IR [43,44]. In addition, Hotamisligil et al., 1999 and Parvaiz et al., 2011, postulated that various HCV proteins interact with the endoplasmic reticulum and mitochondria induces oxidative stress with the concomitant up regulation of inflammatory cytokines [44,45]. Especially, TNF-α. TNF-α was found to be strongly down regulates the insulin signaling mechanisms by blocking the phosphorylation of key molecules Insulin receptor Substrates (IRS) and hampers the glucose transporter (GLUT4) translocation for the glucose molecule across the plasma membrane of the host cell. Moreover, Eslam et al., 2011, reported that HCV interacts with lipid metabolism leading to steatosis, causing wide adipocytokines changes and impairs glucose metabolism leading to increased prevalence of insulin resistance (IR) and T 2DM [31].

Finally, we conclude that patients with chronic HCV Who are candidates to antiviral therapy with PEG-IFN + RBV must be checked for IR before being started on treatment since HOMA-IR index represents a prognostic value for SVR in these patients, regardless of their HCV genotypes.

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