INTRAHEPATIC DETECTION OF INSULIN RECEPTOR SUBSTRATE 2 IN CHRONIC HEPATITIS C PATIENTS

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ABSTRACT

Objective: To detect hepatic insulin receptor substrate 2 in chronic hepatitis C patients.
Study Design: Comparative study.
Place and Duration of Study: Center for research in experimental and applied medicine (CREAM), Department of Biochemistry and Molecular Biology, Army Medical College and Holy Family Hospital Rawalpindi, from Dec 2011 to Nov 2012. Diagnosed patients of chronic hepatitis C were included in the study. Known cases of diabetes mellitus, patients with pancreatic disease and liver pathology other than hepatitis C were excluded from the study.
Material and Methods: Twenty seropositive non diabetic HCV infected patients and 10 control subjects were recruited. Liver biopsy specimen was obtained from seropositive HCV patients while blood samples were obtained from controls as biopsy sample was not possible from normal controls. Both types of specimens were studied for detection of insulin receptor substrate 2 (IRS-2).
Results: No alteration in the content of insulin receptor substrate 2 in both seropositive patients and control samples were detected.
Conclusion: Hepatitis C virus has no effect on insulin receptor substrate 2 content thus indicating absence of hepatic insulin resistance in patients with HCV infection.

Keywords: Hepatitis C Virus (HCV), Insulin receptor substrate 2 (IRS-2), Insulin resistance.

INTRODUCTION

Hepatitis C infection has emerged as a global epidemic affecting 170-200 million individual world widely[1] and 17 million of the Pakistani population[2]. It affects 3,000,000 to 4,000,000 people every year. The use of contaminated needles and instruments in medical practice, unsafe blood and blood product transfusion, intravenous drug abuse, use of unsterilized instruments by barbers and quacks are the most common routes of transmission of hepatitis C virus (HCV) in Pakistan[3]. Hepatitis C infection is a chronic and a multifaceted disease that progresses very slowly. It takes decades for the development of cirrhosis and out of the infected patients, almost 20% develop complications like hepatocellular carcinoma[4]. Insulin can exert its effect after binding to the extracellular portion of the insulin receptor. This receptor has tyrosine kinase activity. After activation of the receptor, phosphorylation of the insulin receptor substrate-2 (IRS-2) occurs which later activates a cascade of different sets of proteins like phosphoinositide 3 kinase (PI3K) and Akt. This causes translocation of glucose transporter 4 (GLUT 4) and ultimately glucose uptake by the hepatocytes[5]. Various proteins of HCV hamper insulin signalling by modulation of cellular gene expression and ultimately leading to diabetes mellitus[6]. Various mechanisms are involved in inducing insulin resistance by upregulating inflammatory cytokine tumour necrosis factor - α (TNF – α), hypophosphorylating IRS - 1 and IRS - 2, phosphorylating Akt and up - regulating gluconeogenic genes[7]. Some of these complications are genotype dependent, evident by the high prevalence of fatty liver in genotype[3]. Thus it directly and indirectly effects the lipid and glucose metabolism[8]. Finally impaired IRS-2

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signalling due to chronic HCV infection leads to insulin resistance and subsequently type 2 DM and hepatic fibrosis[9].

MATERIAL AND METHODS

A comparative study of 20 seropositive non diabetic HCV infected patients and 10 control subjects was conducted at CREAM in the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi from December 2011 to November 2012. Diagnosed patients of chronic hepatitis C were included in the study while known cases of diabetes mellitus, patients with pancreatic disease and liver pathology other than hepatitis C were excluded from the study. After approval from the ethics committee, written informed consent was obtained from the patients. Liver biopsy specimen was obtained from 20 seropositive non diabetic HCV infected subjects. One portion was sent for routine histopathological workup and the remaining biopsy specimen was immediately immersed in liquid nitrogen and then stored at -80°C. Venous blood was taken from controls and collected in ethyldiamine tetra acetic acid (EDTA) tubes. Ribonucleic acid (RNA) was extracted from liver biopsy samples and blood. Following steps were carried out for detection of IRS-2 content.

The primers specific for IRS-2 were designed using Primer 3 Output and then compared with Oligocalc and E-polymerase chain reaction (E-PCR).

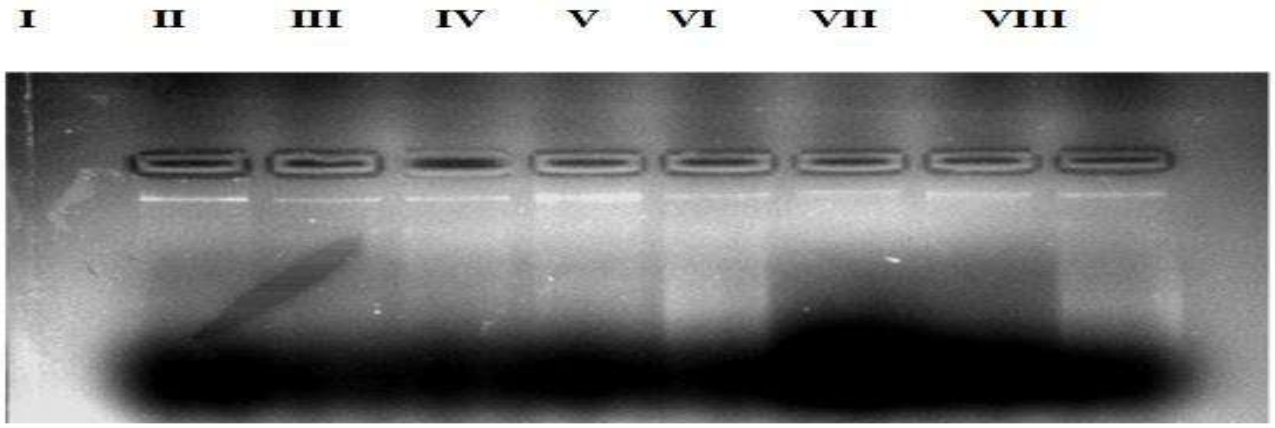

Figure-1: 2% Agarose gel showing the detection of RNA of HCV infected patients. Well I, II, III, IV, V, VI, VII and VIII showing RNA of P1, P2, P3, P4, P5, P6, P7 and P8 respectively.

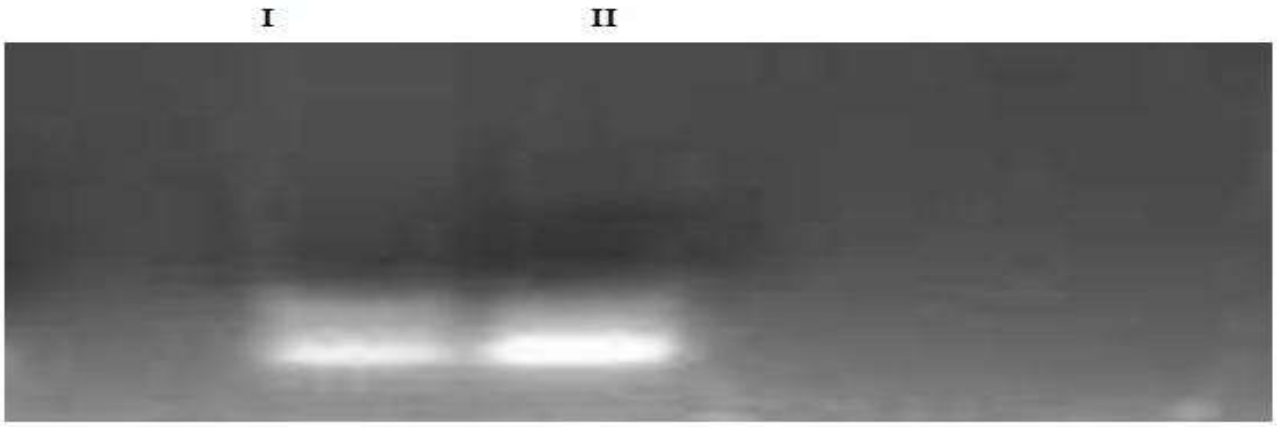

Figure-2: 2% Agarose gel showing cDNA, synthesized from RNA extracted from HCV patients. Well no. I and II showing cDNA of P6 and P7.

Oligo	start	len	tm	gc%
	any	3' seq		
Left Primer	105	19	59.90	57.89
	8.00	2.00 c		
	tacctgcgcaagcagaag			

cDNA was then stored at - 80°c till PCR standardization.

RNA template\ cDNA X ng was used in the reaction mixture. Buffer 1X, $MgCl_2$ 1.7mM, dNTPs 2mM, 1pmol of Forward and Reverse

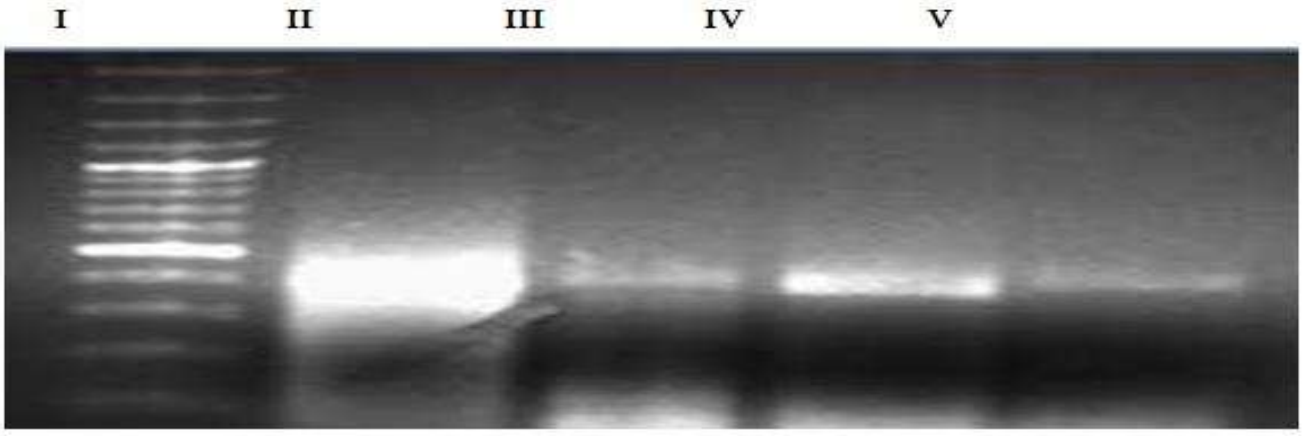

Figure-3: PCR based detection of IRS-2 content when visualized on 2% agarose gel showing the detection of IRS-2 in well II, III and IV of controls. Well I showing marker based of 100-1500 bp.

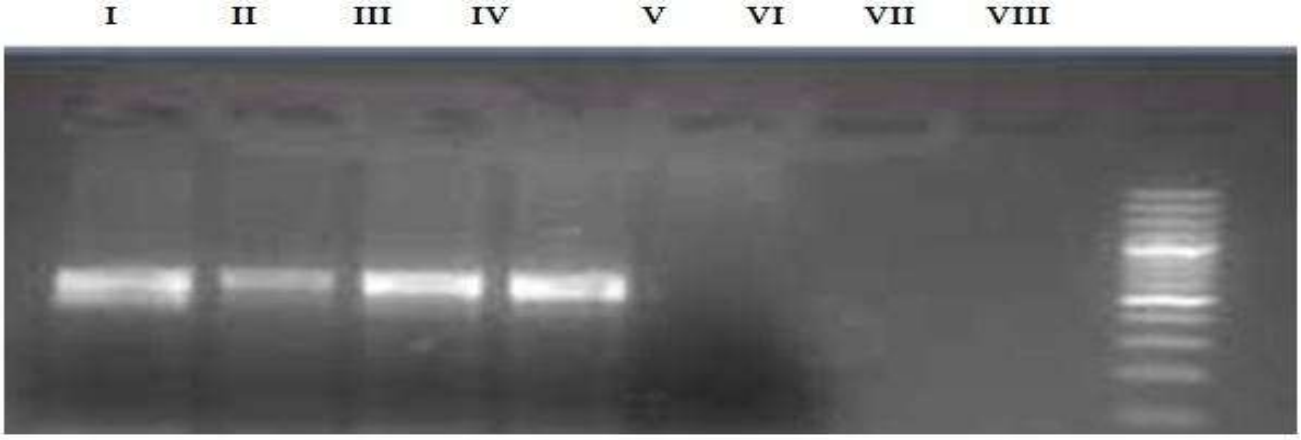

Figure-4: PCR based detection of IRS-2 content when visualized on 2% agarose gel showing the detection of IRS-2 in well I, II, III and IV of P1, P2, P3 and P4 respectively. Well VIII showing marker based of 100-1500 bp.

Right Primer	305	20	59.99	50.00
	3.00	2.00		
	ttgatgttcaggcagcagtc			
Sequence size:	4018			

RNA extraction (Gene Jet RNA Purification Kit, Fermentas) and complementary doxyribonucleic acid (cDNA synthesis (RevertAid Premium First Strand DNA, Fermentas) was carried out as per manufacturer's protocol. The extracted RNA and synthesized primer, was added. Auto claved distilled water X µl and Taq polymerase 1 unit was added. The PCR was optimized through polymerase chain reaction technique on corbet Inc. PCR machine. The following parameters were optimized through series of reaction;

- Hot Start 95°c for 6min.
- Denaturation 94°c for 45 sec.
- Annealing 60.5°c for45 sec.

- Extension 72°c for1.3 min.
- Final Elongation 72°c for 10 min.
- Total Cycles 35.
- Hold 4°c for infinite.

RESULTS

Total RNA was extracted from the biopsy and serum samples, using Gene Jet RNA purification kit. Samples were meticulously handled to reduce contamination. We examined whether HCV infection affects the cellular levels of IRS-2, one of the major substrates for insulin signaling mechanism. Fig-1 shows the extracted RNA in HCV infected patients.

First strand cDNA was synthesized from purified RNA, using Revert Aid Premium First Strand cDNA Synthesis Kit. Two percent agarose gel was made to visualize the cDNA synthesized from the extracted RNA on ultravoilet (UV) illuminator. Fig-2 shows cDNA detection in HCV infected patients.

Synthesis of cDNA was followed by standardization of PCR and finally content of IRS-2 was detected by PCR.

After above mentioned detail it is concluded that the content of IRS-2 was not altered in seropositive non diabetic HCV patients. Thus it is less likely that HCV virus causes insulin resistance in the early years of infection.

Fig 3 and 4 shows PCR based detection of IRS-2 in control subjects and PCR based detection of IRS-2 in HCV infected patients.

DISCUSSION

Chronic viral hepatitis is a major health problem caused by hepatitis B virus (HBV) or hepatitis C virus (HCV). Moreover several studies have shown that HCV is associated with increased incidence of type 2 diabetes mellitus[10-13]. Development of HCV induced insulin resistance is a highly complex mechanism that is still not clear as insulin resistance is limited to the organs or tissues that are infected by HCV. Insulin resistance is one of the major risk factors for the development of type 2 DM. Therefore this mechanism needs to be investigated. The principle findings of our study are that HCV infection did not alter the IRS-2 content thus IRS-2 levels remained unchanged in chronic hepatitis C infection as shown in a recent study conducted by Rhul et al[14]. Our findings are in concordance with the study conducted by Aytug et al in which they found that insulin receptor and IRS-1 content was not changed, while phosphorylation of IRS-1, PI3k and Akt was impaired[15]. Our findings are also supported by Bernsmeier et al in which they showed that insulin signaling is not impaired in all the chronic hepatitis C patients[16]. Moreover it was also in accordance with another study by Hsieh et al, in which mRNA levels were not altered by HCV E2 protein however IRS -1 protein level was reduced[17].

HCV had no effect on the IRS-2 content as shown in our study. This might be because of the difference in HCV genotypes, as most common genotype in our part is 3a and 3b or may be the shorter duration of the disease another factor, as our patients had a disease course of about 2-5 years.

CONCLUSION

Hepatitis C virus has no effect on insulin receptor substrate 2 content thus indicating absence of hepatic insulin resistance in this study group with HCV infection.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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