Original Article

Liver Fibrosis in Chronic Hepatitis C Patients

Relation of Liver Fibrosis Assessed by Elastography with Glycemia in Chronic Hepatitis C Patients

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ABSTRACT

Objective: HCV infection is a major cause of liver fibrosis that may leads to glucose dysregulation. Elastography is a relatively new, non-invasive technique for assessment of liver stiffness. Present study aimed to find out the correlation of degree of liver stiffness with glycemia in chronic HCV patients.

Study Design: Cross-Sectional Study

Place and Duration of Study: This study was conducted at the Institute of Basic Medical Sciences of Dow University of Health Sciences, Karachi for one year.

Materials and Methods: Patients were recruited by convenient sampling technique from the hepatitis clinic and medical ward of Ruth Pfau Civil Hospital, Karachi. A total of ninety participants were inducted. Among them, sixty were HCV related chronic liver disease patients (Group B) of 30-60 years of age of either gender while thirty were age and sex matched healthy control subjects (Group A). Patients not fulfilling the inclusion criteria or not willing to participate were excluded. Fasting blood glucose all participants were estimated. Based on Elastography findings HCV patients were sub-grouped into B1 (METAVIR grade FI and F2) and B2 (MATAVIR grade F3 and F4). Data entered and analyzed by SPSS version 20.0. and presented as frequency (n; %) and mean ±SD. One-way ANOVA, Post Hoc Tuckey's test and Pearson's correlation test were applied where appropriate. A p-value of <0.05 was considered as statistically significant.

Results: Demographic and anthropometric characteristics of the study participants showed non-significant difference compared to control. Liver stiffness was found to be significant raised in advance fibrosis compared to control and early fibrosis group. Patients with advanced fibrosis had significant raised FBG compared to control (p = 0.002) and early fibrosis patients (p = 0.004). Pearson's correlation test revealed significant moderate positive relation (r=0.393, R^2=0.155) of FBG with liver fibrosis (p= 0.000).

Conclusion: Liver stiffness as assessed by elastography has significant positive correlation with glycaemia in chronic HCV patients.

Key Words: Chronic Hepatitis C, Liver Fibrosis, Shear wave Elastography

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INTRODUCTION

revalence of hepatitis C infection is increasing at an alarming rate effecting nearly 185 million people worldwide[1]. HCV infection rate in Pakistan has also reached an epidemic proportion with nearly 10 million people in the country lived with this virus.

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According to an estimate of WHO, Pakistan stands second after Egypt according to number of people infected with this virus[2].

HCV once considered as hepatotropic virus affecting and limited to the liver, growing body of evidences now pointed out role of this virus in manifestation of certain extra hepatic diseases[3]. Though exact prevalence of these extra hepatic manifestation was not known, Gill K et al, reported nearly 40-74% of HCV patients had some of the extrahepatic manifestations ranging from sever fatigue to certain form of lymphoma[3].

Dysregulation of blood glucose is one of the extra hepatic manifestations of CHC[4]. Epidemiological studies reported nearly 14-33% of CHC patients developed type 2 diabetes (T2DM)[5]. Numerous mechanisms have been suggested for T2DM development in CHC patients that ranges from direct infection of beta cells of pancreas to the development of insulin resistance due to hepatic fibrosis and cirrhosis[6]. Studies reported increased frequency of T2DM with every rise in fibrosis score of HCV patients with an OR

of 3.83. Cirrhosis is the final stage of liver fibrosis and liver inflammation, may reduce uptake of glucose by hepatic cells thus affects the glucose metabolism[7].

There are various methods for evaluating and grading liver fibrosis. It ranges from invasive procedure like liver biopsy to non-invasive evaluation of fibrosis by serological panels, mathematical calculations of certain serum fibrosis markers and clinical data to imaging techniques [8,9].

In contrast to liver biopsy which is painful and determine very small volume of hepatic parenchyma with inter and intra observer variability, SWE is one of the non-invasive modality for the assessment of liver fibrosis and can examine 100 times bigger volume of liver tissue (10). Meta-analysis conducted on diagnostic accuracy of noninvasive modalities suggested high accuracy of SWE and clinically useful for fibrosis F≥2 and identical for liver cirrhosis as compared to other non-invasive modalities such as RTE, ARFI, TE [11]. Furthermore, unlike transient elastography, which consists of a vibrator producing shear waves, the latter can perform a conventional ultrasound at the same time. Therefore, shear wave elastography (SWE) may be preferred, because sensitivity and specificity for diagnosing hepatic cirrhosis was nearly 90% and for hepatic fibrosis it was 70-80% [12].

Present study thus evaluated the relation of serum glucose with severity of liver fibrosis as assessed by SWE in order to validate the findings of earlier studies in which liver fibrosis was graded by other modalities. SWE Elastography is a new technique for measuring the liver stiffness in Kpa and graded according to MATAVIR classification in to F1, F2, F3 and F4. Present study thus aimed to find out the correlation of liver stiffness/fibrosis as assessed by SWE elastoraphy with glycemic status of non-diabetic chronic HCV patients.

MATERIALS AND METHODS

This descriptive, cross sectional study was conducted in the Institute of Basic Medical Sciences of Dow University of Health Sciences. Study was conducted after ethical approval from the institutional review board of DUHS. Patients were selected from the hepatitis clinic and medical ward of Ruth Pfau Civil Hospital, Karachi. Sample size was calculated by PASS version II sample size calculator taking confidence interval and power 99%. A total of ninety (n=90) participants were inducted in this study by convenient sampling technique. Among them, sixty (n=60) were HCV related chronic liver disease patients (Group B) of 30-60 years of age of both gender while thirty (n=30) were age and sex matched healthy subjects (Group A). Subjects with history of hypertension, smoking, alcohol, ascities, pregnancy, co-infection with other types of hepatitis viruses, patients with history of thyroid diseases and persons not willing to participate were excluded. Elastography of the selected patients (n=60) and controls (n=30) were performed in the radiology department of Ruth Pfau Civil Hospital, Karachi. Liver stiffness was expressed in Kpa and grouped according to METAVIR classification. According to liver stiffness value patients were graded into grade1; 6.2Kpa (5.3-7.1), grade II; 7.6Kpa (7.0-8.5), grade III; 10.0Kpa (9.5-11.6) and grade IV; 15.6Kpa (13.1-18.8). These patients were further sub grouped into B1 (patients with elastography METAVIR fibrosis grade FI and F2) and Group B2 patients with (elastography MATAVIR fibrosis grade F3 and F4). Blood samples from each participant were collected after 12 hours of fasting for estimation of fasting blood glucose level. Elastography was done after blood collection. Data was entered and analyzed by SPSS version 20.0. and presented as frequency (n; %) and mean ±SD. One-way ANOVA was applied to compare the mean (±SD) among group variable with significant difference in ANOVA was analyzed by Post Hoc Tuckey's test. Pearson's correlation test was performed by to find out the association of liver stiffness with glycemic status. A p-value of <0.05 was considered as statistically significant.

RESULTS

A total of ninety participants including thirty control (n=30) and sixty (n=60) known HCV patients were inducted in the study. Demographic and anthropometric characteristics of the study participants are shown in table 1. Comparison among groups revealed non-significant difference in demographic and anthropometric characteristics of HCV patients compared to control.

Mean (±SD) values of liver fibrosis among hepatitis C patients in group B1 and B2 and control were assessed by shear wave ultrasound and categorized according to METAVIR classification.

A significant difference in mean (±SD) of liver fibrosis among group was observed by application of one way ANOVA test (Figure 1). Posthoc Tuckey's test revealed significant difference in mean ±SD of liver fibrosis in late fibrosis group compared to control (group A) and patients in early fibrosis group (group B1).

Liver stiffness among the study groups by Shear wave Elastography (mean±SD)

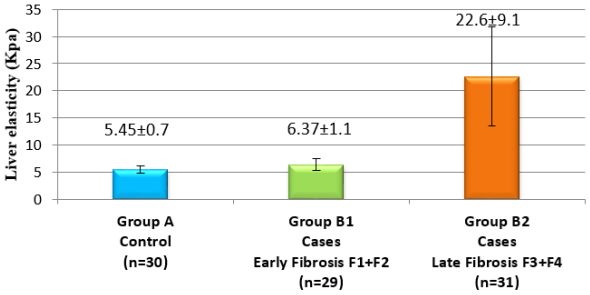

Figure No.1: Comparison of liver stiffness among study group by one-way ANOVA

Table No.1: Comparison of demographic and anthropometric characteristics among study participants

	Group A	Group B 1	Group B 2	F-value	P-Value
Variables	**Control (n=30)**	**Early Fibrosis F1+F2 (n=29)**	**Late Fibrosis F3+F4 (n=31)**		
Age (years)	45.8±8.2	45.7±11.0	50.4±7.0	2.74	0.070
Male	14 (46.7%)	14 (48.3%)	19 (61.3%)	1.57	0.455
Female	16 (53.3%)	15 (15.7%)	12 (38.7%)		
Height (m)	1.61±0.04	1.61±0.02	1.61±0.02	0.44	0.640
Weight (kg)	66.2±11.9	68.3±2.6	67.3±12.3	0.33	0.719
BMI	25.5 ± 0.8	26.0±0.6	26.1±0.7	0.33	0.715

A significant difference in fasting blood glucose (FBG) was observed by application of ANOVA test revealed significant p-value = 0.001, as shown in Table 2. Application of Post Hoc Tukey's test revealed patients in early fibrosis (group B1) had non-significant difference (p= 0.954) whereas patients in late fibrosis (group B2) had significant raised FBG compared to group A (p = 0.002). Late fibrosis patients (group B2) had significantly raised level of fasting blood glucose compared to early fibrosis (Group B1), (p = 0.004) Table 2.

Table No.2: Comparison of (mean ±SD) FBS Level among study groups by one-way ANOVA and Pot Hoc Tuckey's test

	Group A	Group B 1	Group B 2	F-value	P-Value
Variables	**Control (n=30)**	**Early Fibrosis F1+F2 (n=29)**	**Late Fibrosis F3+F4 (n=31)**		
FBS (mg/dl)	88.1±21.6	90.2±20.3	113.0±35.6	7.97	0.001

p-value < 0.05 is significant

The correlation between FBS level and liver fibrosis (shear wave average) shown in table 3 revealed significant moderate positive relation (r=0.393, R^2=0.155) with p-value 0.000. R^2 shows 15% variation in FBS was explained by liver fibrosis as shown table 3.

Table No.3: Correlation of FBS in HCV infected patients with liver Fibrosis

Parameter	r-value	R^2-Value	% of determination	p-value
FBS mg/dl	0.393	0.155	15%	0.000**

DISCUSSION

DM and Hepatitis C are chronic diseases, prevalent throughout the world[1]. Present study revealed the relation of blood glucose with liver stiffness in HCV infected patients. A significant positive association of fating blood glucose with severity of liver fibrosis was observed.

Considered earlier as hepatotropic virus, in the recent past this virus was found to have increased propensity to infect extrahepatic tissues as well. Studies reported increased propensity of HCV virus to dysregulate glucose metabolism. Various mechanisms of glucose dysregulation in the literature have been reported that predispose HCV patients to diabetes, both directly as well as through indirect mechanism. Infection of β-cell of pancreas by HCV effects the insulin signaling pathway, ultimately results in DM[13]. Besides this direct effect, in some studies, presence of fibrosis, has been shown to be an independent risk factor that contributes to the progression to T2D[14]. Seventy to eighty percent of HCV cirrhotic have been reported to have glucose intolerance, and 50% of these cirrhotics have developed DM[15]. The rates of development of diabetes has been reported to be more increased on the background of higher grades of hepatic fibrosis, steatosis, or cirrhosi[16]. Whereas some literatures like the study done by Hanchanale P et al, reported increased frequency of diabetes even in non-cirrhotic chronic HCV patients and suggested pathogenesis of DM in HCV patients is multifactorial initiated in pre-cirrhotic stage leading to abnormal glucose metabolism and insulin resistance[17] The inconsistency in published reports between relation of diabetes with presence of cirrhosis in HCV patients may be because of subtle variations among studies in study design, patient selection as well as difference in modality used for assessment and grading of liver fibrosis.

Like above mentioned studies, numerous other published studies that explored the relation of hepatitis C virus with glycemic status of the patients.[18,19] used different modality for assessment of liver fibrosis. For example, Saad Y et al, find out the relation of blood glucose and fibrosis in liver. Authors in that study evaluated liver fibrosis in Egyptian diabetic patients with hepatitis C virus[20]. In other studies, similar relation of glucose with liver fibrosis was reported. The studies conducted by Sabry HS et al, Turner BJ et al, and Chen Yet al, also explored the relation between liver fibrosis with glycemic status of the chronic HCV patients. In these studies, however liver fibrosis is assessed by liver biopsy and APRI and FIB4 respectively[21,22,23]. These studies also despite of use of varied methodology for the assessment of liver fibrosis unanimously reported the association of liver fibrosis with glycemic status of the patients.

Liver biopsy is the gold standard in the diagnosis and staging of liver fibrosis. But it evaluates only 1/50000 of the liver parenchyma. It is invasive and in rare instances leads to severe complications[10]. Because of the imperfect nature of liver biopsies, over the last

several years there has been a growing trend to validate non-invasive tools to diagnose and stage liver fibrosis. Laboratory marker like (Alkaline aminotransferase platelet ratio index (APRI)) has been shown to have some value but is inferior to liver biopsy[24]. Alternatively, magnetic resonance has been used for elasticity imaging. Magnetic Resonance Elastography, even though promising, has some disadvantages. Aside from the significant cost, it cannot be performed in a liver with iron overload. It also required longer examination times compared to ultrasound elastography[25]. Ultrasound elastography has been validated and has been shown in many studies to have almost similar sensitivity and specificity to liver biopsies[26].

Present study was conducted in an attempt to validate the findings of earlier studies. Present study is unique as in this study liver fibrosis is evaluated by non-invasive, sheer wave ultra-sonographic technique that measures the liver stiffness in Kpa. Values of liver stiffness in Kpa is categorized and considered equivalent to the various grades of METAVIR classification system[26]. METAVIR scoring system is used to assess the extent of inflammation and fibrosis by histopathological evaluation in a liver biopsy of patients with hepatitis C. The grade indicates the activity or degree of inflammation while the stage represents the amount of fibrosis or scarring[27]. A significant positive correlation of liver stiffness with glycemia in the present study is consistent with the available literatures however it serves as a mean to look this old relation through a new window of non-invasive procedure.

CONCLUSION

Liver stiffness as assessed by elastography has significant positive correlation with glycemia in chronic HCV patients. Thus validated the findings of earlier studies evaluated liver fibrosis by other methodology.

Author's Contribution:

Concept & Design of Study:	Talat Samreen
Drafting:	Sagheer Ahmed, Syed Munawer Alam
Data Analysis:	Syeda Asia Perveen
Revisiting Critically:	Talat Samreen, Sagheer Ahmed
Final Approval of version:	Talat Samreen

Conflict of Interest: The study has no conflict of interest to declare by any author.

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