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Association of hepatitis G with liver dysfunction in treatment responder hepatitis C patients

[1] Uzma Najam, [2] Syed Tousif Ahmed, [3] Rubina Ghani, [4] Nousheen Zehra, [5] Ahsan Suleman
[1] Lecturer, department of Physiology, Ziauddin University, Pakistan
[2] Professor and Head, Department of Physiology, Ziauddin University, Pakistan
[3] Associate Professor, Department of Biochemistry, Baqai University, Karachi, Pakistan
[4] Assistant Professor, Department of CHS, Ziauddin University, Pakistan
[5] MBBS, Liaquat National Medical College, Pakistan

Abstract
Background: Hepatitis G (HGV) is a newly discovered hepatotropic virus but there is controversy about the role of HGV infection in the pathogenesis of chronic liver disease. There is no information about the frequency of hepatitis G with liver dysfunction in treatment responders hepatitis C patients. The objective of study was to assess liver functions of treatment responder hepatitis C patients and to detect the presence of GBV-C/HGV virus in these patients. The association of GBV-C/HGV with liver dysfunction and its frequency in treatment responder hepatitis C patients was also determined.
Methods: 250 treatment responder hepatitis C patients with liver dysfunction were recruited for this cross-sectional study. LFTs were checked and HGV RNA was investigated in serum samples by reverse transcription and polymerase chain reaction amplification. LFTs of sero-positive and sero-negative HGV patients were compared. Data was analyzed on SPSS version 20.
Results: A total of 250 treatment responder hepatitis C patients were evaluated with a mean age 44 ± 5.55. Males constituted 38.4 % (N=96) while female constituted 61.6% (N=154) of our study group. Out of total study subjects 18 (7.2%) of the study subjects were Hepatitis G positive. Highly significant difference of mean LFTs was found between hepatitis G positive and negative patients with p-value < 0.01. We found highly significant and positive association of Hepatitis G with total billirubin, direct billirubin, indirect billirubin, GGT and APTT (p-value < 0.01 at 95% CI).
Conclusion: Liver dysfunction in treatment responder hepatitis C patients is found to be associated with novel Hepatitis G virus.

Keywords: Liver function tests, Hepatitis G, Treatment responder hepatitis C patients

Introduction
The major gland of the body is liver having reddish-brown colour and comprises of four lobes. The interior of the liver is packed with incalculable minute cylinders called hepatic lobules which are bordered by glowing sheets of cuboidal cells called hepatocytes. (Saladin, 2011). There are numerous factors for instance age, gender, body surface area and nutrition affect the size of liver. Anatomically, portal vein (PV) has complicated relation with the liver structural design. The diameter and flow of the PV is influenced by any pathology in the hepatic vasculature and parenchyma which leads to portal hypertension and cirrhosis (Siddiqui *et al.*, 2014). Infection or any other associated diseases originate changes in liver function, producing an imbalance escorting to irreversible severe hepatic diseases, such as liver cirrhosis, liver cancer and hepatitis (Gupta *et al.*, 2014). The liver serves as a power house for metabolism which stores and produces numerous essential products for instance glucose, amino acids and plasma proteins (Koeppen and Stanton, 2009). Almost all the plasma proteins which account about 90 per cent except the gamma globulins are synthesized via hepatic cells. Substances fashioned in the liver which are essential in the coagulation process include fibrinogen, prothrombin accelerator globulin, Factor VII and several other significant factors. Vitamin K is requisite by the metabolic processes of the liver for the production of several of these substances, in particular prothrombin and Factors VII, IX, and X (Hall, 2010). The liver also executes an extensive range of functions not associated with digestion**.** Metabolic processing of the major categories of nutrients (carbohydrates, proteins, and lipids) comes about after their absorption from the digestive system. Detoxification of drugs, hormones and other foreign compounds occur in liver (Sherwood and Pysiology, 2010).
Universally in clinical practice, the evaluation of liver function tests (LFTs) is widely practiced for the screening of liver disease, to scrutinize the prognosis of diseases, and to inspect the effects of hepatotoxic drugs. The mainly well-known LFTs embrace bilirubin, alkaline phosphatase, the serum aminotransferases, albumin, and prothrombin time. Aminotransferases (ALT and AST) are the key markers that signify hepatic injury by concluding intracellular hepatic enzymes concentration in blood. Worldwide, hepatic function and cholestasis is markedly demonstrated by alkaline phosphatase (AP), γ-glutamyl transpeptidase (GGT), and bilirubin. Synthetic functions of liver are manifested by albumin and prothrombin (Harris, 2005) [11]. Liver diseases are the major cause of morbidity and mortality (W. Ray Kim *et al*, 2002).Liver disease is now the fifth most common cause of death after heart disease, stroke, chest disease and cancer (Roger Williams *et al*, 2006). Liver dysfunction in critically ill patients is frequent and is associated with increased mortality (S Musa *et al*, 2009). The burden of liver diseases is estimated by calculating the incidence and prevalence of cirrhosis and primary liver cancer (Blachier *et al*, 2013). New Global

Burden of Disease estimates for liver cirrhosis, propose that cirrhosis is the root cause over a million deaths in 2010 which was approximately 2% of all deaths, with an additional million due to liver cancer and acute hepatitis (Byass, 2014). Viral hepatitis is a crucial worldwide health problem. Presently, six distinctive types of hepatitis viruses have been recognized and named as hepatitis A, B, C, D, E and GBV-C/G viruses (Bosan *et al*., 2010). Hepatitis C virus (HCV) infection is a foremost worldwide health issue. Earlier global burden of disease approximate published by the World Health Organization (WHO) embrace only burden from acute HCV infection (Hanafiah *et al.*, 2013). Pakistan has the second topmost prevalence rate of hepatitis C ranging from 4.5% to 8%. Studies in Pakistan on small embattled groups comprising blood donors, health professionals, drug abusers and chronic liver disease patients point out that the prevalence of hepatitis C is up to 40% (Fatima *et al*., 2015). In 1995-96, quite a few novel human RNA viruses were recognized and predictable to be distinct from other human hepatitis viruses (A, B, C, D, E). They were partially distinguished to cause acute and chronic hepatitis (Waqar *et al.*, 2002). GBV-C is a member of the Flaviviridae family and is phylogenetically correlated to hepatitis C virus (Leary *et al.*, 1996). While comparing the genomes of GBV-C, GBV-A, GBV-B and HGV, it was demonstrated that their RNA had not stand a more than 32% resemblance. So this characteristic feature gave the strength to support the hypothesis that they were independent (Reshetnyak *et al*., 2008) [10]. It is difficult to establish the frequency of HGV infection, but studies recommend that in most developed countries, the prevalence is 1% - 4% in healthy blood donors while 5% -13% have anti-E2 antibody which is an early indicator of infection(Stapleton *et al.*, 2011). Hepatitis G is a blood- borne virus which can be transmitted parenterally and vertically (Reshetnyak *et al*., 2008) [10]. Like HBV and HCV, HGV can be sexually transmitted which is verified by the high prevalence of HGV-RNA in homosexuals and prostitutes with 13.4% to 63% and 13.9% to 24.8% respectively. The prevalence of HGV in blood donors is higher approaching 20% in few regions of world (Stapleton *et al.*, 2011). By current investigation, it has been reported that much higher HGV infection rate of 30% to 36% is found with HIV/HCV co-infection (Feng *et al.*, 2011).

However, as not much literature is available on the relationship of HGV with liver dysfunction, the current study was conducted.

Method

A cross sectional study was designed in which 250 treatment responder hepatitis C patients, who fulfilled the inclusion and exclusion criteria were selected. Patients were recruited from Jinnah Post Medical College & Ziauddin University Hospital Karachi. Patients were informed of the study and written, signed consent was taken from them. Demographic profile including age, gender, ethnicity and socioeconomic status was collected from each subject through a questionnaire

Inclusion Criteria

Study participants were selected Treatment responders Hepatitis C patients (undetectable HCV RNA in the serum after 24 weeks of post treatment follow up)

Exclusion Criteria

Patients with relapse cases of hepatitis C, who had pregnancy, renal diseases, history of hepatotoxic drugs used in past 3 months, congestive cardiac failure, Diabetes Mellitus, fatty liver disease and those who were alcohol addicts, excluded from the study. Participants were asked to submit a written consent of voluntary participation and demographic data was obtained through a questionnaire. Sampling was done after taking approval from Ethical Review Committee, Ziauddin University

Parameters to Assess Hepatotoxicity

Assessment of liver function test: Serum was separated by centrifuging blood at 2500 rpm for 10 minutes and the levels of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (γ-GT), prothrombin time (PT), activated partial thromboplastin time (APTT), total protein and albumin were analyzed by using a commercially available enzymatic kit.

Methodology of Hepatitis G Virus Detection

RNA Extraction

Total RNA was extracted from 150µl of serum using Epicenter viral RNA extraction kit (Epicenter, USA), following manufacturer's instructions. Briefly, proteins were precipitated and RNA was carefully separated from the organic phase and allowed to precipitate in the presence of isopropanolol, also added at this step as a carrier molecule. RNA pellet was then washed twice with 70% ethanol. After ethanol evaporation RNA pellet was reconstituted in 10ul of DEPC-treated water containing RNAse inhibitor and stored at -70oC till further amplification. HGV RNA was amplified by RT-PCR and nested PCR from highly conserved region of the viral genome, i.e. 5'-untranslated region (5'-UTR). HGV RNA was reverse transcribed by RT-PCR method, using anti sense primers.

Gel Electrophoresis

Amplified products (210bp) were analyzed by electrophoresis on 2% agarose gel and visualized under UV-trans-illuminator.

Statistical Analysis

All data was entered and analyzed using SPSS Version 20. For categorical variables, frequencies and percentages were calculated. Mean and standard deviation were calculated for numerical variables. Pooled t-test was applied for finding difference between two numerical data. Chi-Square test and Log Regression test were applied to find association between categorical variables. P-value < 0.05 was considered statistically significant.

Results

A total of 250 treatment responder hepatitis C patients were evaluated. Subjects belonged to different ethnicities and the mean age group was 44 ± 5.55years. Males constituted 38.4 % (N=96) while female constituted 61.6% (N=154) in our study group. Mean LFTs of all subjects is mentioned in table 1. Mean total bilirubin, direct bilirubin and indirect bilirubin were 0.772 ± 0.19 mg/dl, 0.308 ± 0.99 mg/dl and 0.496 ± 0.55

mg/dl respectively. Mean ALT was 81.97 ± 38.03 units/L while mean AST was 32.44 ± 7.58 units/L. in our study group mean alkaline phosphatase was 221.67 ± 51.2 units/L while mean gamma-GT was found to be 34.23 ± 7.58 units/L. Mean PT was 17.2 ± 2.14 sec whereas mean APTT was 35.94 ± 4.49 sec. Mean total protein and albumin was 5.25 ± 0.44 g/dl 2.64 ± 0.53 g/dl respectively (Table-1).

Out of total study subjects 18 (7.2%) of the study subjects were Hepatitis G positive (table-2). Pooled t- test was applied to find out difference in mean of two independent groups. Highly significant difference of mean LFTs was found between hepatitis G positive and negative patients with p-value < 0.01 (Table-3).

To find out association of LFTs with hepatitis G positive patients, chi-square was used. Total bilirubin, direct bilirubin, indirect bilirubin, AST, GGT, and APTT are found to be associated with hepatitis G with highly significant p-value < 0.01 (table-4)

Log regression was applied to find whether the association between LFTs and hepatitis G was positive or negative. We found highly significant and positive association of Hepatitis G with total bilirubin, direct bilirubin, indirect bilirubin, GGT and APTT (p-value < 0.01 at 95% CI) shown in (table-5).

Table 1: Left Findings in Our Study Subjects

Characteristics	Mean±SD
Total Bilirubin mg/dl	0.772 ± 0.19
Direct Bilirubin mg/dl	0.308 ± 0.99
Indirect Bilirubin mg/dl	0.496 ± 0.55
ALT units/L	81.97 ± 38.03
AST units/L	32.44 ± 7.58
Alkaline Phosphatase units/L	221.67 ± 51.2
Gamma GT units/L	34.23 ± 7.58
PT sec	17.2 ± 2.14
APTT sec	35.94 ± 4.49
Total Protein g/dl	5.25 ± 0.44
Albumin g/dl	2.64 ± 0.53

Table 2: Frequency of Hepatitis G in Our Study

Hepatitis G	N = 250	%
Hepatitis G Positive	18	7.2
Hepatitis G Negative	232	92.8

Table 3: Difference in Mean Lefts between Hepatitis G Patients

LFTs	Hepatitis G Positive n = 18	Hepatitis G Negative n = 232	p- value
Total Bilirubin mg/dl	1.07 ± 0.16	0.75 ±0.18	0.001**
Direct Bilirubin mg/dl	0.42 ± 0.07	0.30 ±.10	0.001**
Indirect Bilirubin mg/dl	1.09 ± 1.98	0.45 ± 0.12	0.001**
Alkaline PO_4 units/L	250.78 ± 35.09	219.42 ± 51.62	0.012**
ALT units/L	162 ± 35.38	75.77 ± 30.50	0.001**
AST units/L	37.72 ± 5.95	32.04 ± 4.27	0.001**
Gamma GT units/L	43.50 ± 8.06	33.51 ± 7.07	0.001**
PT sec	20.67 ± 1.71	16.98 ± 1.94	0.001**
APTT sec	42.17 ± 2.85	35.47 ± 4.24	0.001**
Total Protein g/dl	4.61 ± 0.25	5.31 ± 0.42	0.001**
Albumin g/dl	1.95 ± 0.34	2.70 ± 0.50	0.001**

** p-value < 0.01 highly significant

Table 4: Association of Lfts with Hepatitis G

LFTs	Hepatitis G		p- value
	Positive n= 18 (7.2%)	Negative n = 232 (92.8%)	
Total Bilirubin mg/dl	11 (61.11%)	13 (5.6%)	**0.001****
Direct Bilirubin mg/dl	5 (28%)	10 (4.3%)	**0.001****
Indirect Bilirubin mg/dl	7 (39%)	7 (3%)	**0.001****
Alkaline Po4 units/L	8 (44%)	57 (24%)	0.064
ALT* units/L	18 (100%)	232 (100%)	
AST units/L	13 (72%)	98(42%)	**0.014****
Gamma GT units/L	12 (67%)	40 (17.2%)	**0.001****
PT sec	18 (100%)	212 (91%)	0.194
APTT sec	3 (17%)	1 (0.4%)	**0.001****
Total Protein*g/dl	18 (100%)	232 (100%)	
Albumin g/dl	18(100%)	228 (98%)	0.574

*ALT and total protein levels were found to be deranged in all subjects
** p- value < 0.01 highly significant

Table 5: Association of Lfts with Hepatitis G

LFTs	Odds ratio OR	p- value	Confidence Interval (95% CI)
Total Bilirubin mg/dl	26.473	0.001**	8.80 - 79.56
Direct Bilirubin mg/dl	8.53	0.001**	2.54 - 28.64
Indirect Bilirubin mg/dl	20.455	0.001**	61 - 68.58
Alkaline Po4 units/L	2.456	0.71	0.92 - 6.52
AST units/L	3.55	0.19	1.22 - 10.3
Gamma GT units/L	9.6	0.001**	3.40 - 27.09
PT sec	3.6	0.38	0.21 - 61.4
APTT sec	46.2	0.001**	4.52 - 471.3
Albumin g/dl	0.7	0.834	0.03 - 14.1

** p- value < 0.01 highly significant

Discussion

Globally, liver disease is the fifth most common cause of death and the major cause of morbidity and mortality.At the present time, liver dysfunction is frequent and is associated with increased mortality while many possible reasons of liver dysfunction such as viral hepatitis, alcohol use, steatosis or steato-hepatitis, any history of blood transfusion, diabetes, heart disease, thyroid disease and cirrhosis. Hepatotropic viral infections include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV), all of which are moderately prevalent in our population. A recently discovered and identified non A-E virus has been designated the name of hepatitis G virus (HGV) which is a member of the Flaviviridae family having 25% homology with hepatitis C. Based on genomic sequence comparison, HGV is also named as GBV-C which has clearly established transmission modes including mainly blood contamination, vertical and sexual transmission [1]. Uncommon but well documented viral causes of liver failure include HGV, Herpes Simplex and Cytomegalovirus whereas a few data suggest a major pathogenic role of HGV, SEN and TT (transfusion transmitted) viruses [2]. HGV is also responsible for liver cirrhosis and hepatocellular carcinoma [3]. The mean age of our study participants had high proportion of female as compared to male. The most possible cause of predominance of females is receiving blood transfusion at the time of delivery, as a study emphasizes that more than five blood transfusions increases the risk of HGV significantly [1]. Another study conducted in Singapore to find out the prevalence of Hepatitis G in chronic liver disease and it was found out quite similar to our study with female predominance as compared to male [4]. A study in patients with chronic hepatitis C was associated with male sex and they had the history of intravenous drug use [5]. Both of these studies had dominance of male which does not correlate with our study as data suggest that parenteral exposure plays an important role in the transmission of HGV. Our study showed that of the total study participants, 18 (7.2%) of the study subjects were Hepatitis G positive. Our result of HGV infection rate in treatment responder Hepatitis c patient was quite similar to HGV infection in patients with chronic hepatitis C [6] while in Singapore the frequency of HGV in patients with chronic liver disease was found to be low [4]. A study conducted in India which illustrated the prevalence of HGV was as low as compared to my study in chronic liver disease [7]. The detection rate of HGV in blood donors from many countries ranged from 0.5 to 7.4% while in healthy Korean blood donors, the detection rate of HGV was low [8]. In a study which was conducted in Brazil, HGV – RNA was detected in patients with hepato-cellular carcinoma was similar to that reported in Italy while in china the highest frequency was observed [9]. The most probable explanation of HGV occurrence in blood donors is that hepatitis G is a blood- borne virus and its transmission is via parenteral and vertical route while its occurrence in chronic liver patients is due to HGV detection in hepatocytes, peripheral blood lymphocytes and monocytes, vascular endothelial cells and other tissue [10]. Globally liver function tests (LFTs) are essential in clinical practice for screening purpose to rule out liver disease, observe the progression of known disease, and analyze the side effects of potentially hepatotoxic drugs [11]. In our study group we compared the difference in mean LFTs of hepatitis G positive and negative patients and the difference of mean LFTs is found significantly different between the subjects of both groups. A study in HGV infected patients showed elevation of aminotransferases level tend to be limited while patients had modest elevation of alkaline phosphatase and gamma-glutamyltranspeptidase levels [12, 13] and same results were found out in another study which demonstrated increased activity of alanine aminotransferase (ALT) levels in HGV infected subjects with evidence for increase in the activity of alkaline phosphatase and gamma glutamyltranspeptidase level which is consistent with our study group [10]. In a study which was conducted in Barcelona point up the minimal elevation of ALT in HGV infected blood donors whereas no significant difference between HGV infected and non-infected chronic hepatitis C patients was observed in LFTs [6]. This is a novel study regarding analysis of liver dysfunction in chronic hepatitis C patients as no study has been done till yet. In our study group total Bilirubin, direct Bilirubin, indirect Bilirubin, AST, GGT, and APTT are found to be associated with hepatitis G. In Brazil, patients of hepatocellular carcinoma were evaluated and patients with HGV infection was found to be associated with elevated ALT, GGT and APTT [9] which resemble with the findings of our study group. Another study which was conducted in Singapore, in the patients with chronic liver disease were found to be associated with deranged ALT, GGT, APTT and serum albumin [4]. In our study group, we found highly significant and positive association of Hepatitis G with total Bilirubin, direct Bilirubin, indirect Bilirubin, GGT and APTT at 95% CI. In a study with subjects of fulminant hepatitis HGV was detected in 16% cases and found positive association with ALT [10]. A study conducted in Brazil also illustrated positive association with AST in HGV infected patients [9]. Both of these studies are consistent with our study group. A study in patients with

hepatocellular carcinoma demonstrated HGV RNA positivity with statistically significant 9.5 fold at 95% CI elevation in ALT, AST and GGT [14].

Conclusion

The findings of the subjects of our study group conclude that novel hepatitis G virus is found to be associated with liver dysfunction in treatment responder hepatitis C patients. It is advisable to recommend HGV testing in patients with acute, chronic and fulminant hepatitis. In the interest of safety, it is better that each blood bag be screened for HGV rather than risk transferring HGV to blood recipients.

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