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COMPARATIVE EVALUATION OF LFT IN PATIENTS WITH ALCOHOL CONSUMPTION CO-INFECTED WITH BLOOD BORNE VIRUSES

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ABSTRACT

This study aims to assess changes in liver enzyme levels among individuals with liver disease and compare them to healthy individuals to determine the severity and effects of HBV, HCV, HIV, and co-infections. A total of 1,012 serum samples were collected, including 512 from patients with liver disease and 500 from healthy individuals. These samples were screened for HBV, HCV, and HIV markers using ELISA tests. Liver function tests (LFTs) were conducted to measure levels of albumin, bilirubin (total, direct, and indirect), and enzymes such as serum alkaline phosphatase (SAP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase. Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate differences between the groups. A significant increase in liver enzyme levels was observed in HCV-affected patients, with pigmentation noted in those co-infected with HBV, HCV, and HIV. The statistical analysis revealed significant levels of total, direct, and indirect bilirubin, as well as SAP, among liver disease patients compared to healthy individuals. Notably, HCV-positive individuals who consumed alcohol exhibited higher liver enzyme and pigment levels. Additionally, alcohol consumers with HCV and HIV co-infections showed a greater elevation in liver enzymes than non-alcoholic HCV patients. The study indicates significantly elevated liver enzymes in HCV patients, particularly those with co-infections and alcohol consumption. Liver function tests are crucial for assessing disease severity, revealing notable differences between healthy and diseased individuals.

KEYWORDS: Hepatotropic viral infections, Liver enzyme levels, Hepatitis C virus (HCV), Liver function tests (LFTs), Alcohol consumption, Human Disease

INTRODUCTION

Globally, hepatitis and other liver infections are widespread, primarily due to bacterial and viral agents. Cirrhosis results from prolonged liver dysfunction, with viral hepatitis posing a significant public health threat. This condition is mainly caused by six hepatotropic viruses: Hepatitis A virus (HAV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Hepatitis E virus (HEV), and Hepatitis G virus (HGV). Notably, HBV and HCV are the leading causes of chronic liver infections worldwide [1]. In India, HBV and HCV infections are among the most common causes of liver disease [2]. These infections increase the risk of hepatocellular carcinoma, cirrhosis, and liver failure in affected individuals [3]. Over the next two decades, the incidence of cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) is expected to rise significantly among patients with HBV/HCV infections [4,5]. HCV, HBV, and HIV share similar infection mechanisms, making co-infection a frequent occurrence [6]. To date, no clinical survey has accurately quantified the global prevalence of co-infection with HCV and HBV [7]. Co-infection with hepatotropic viruses has become a major concern among HIV-positive individuals [8]. Previous studies have shown that liver disease caused by HCV and hepatitis Delta agents can progress more aggressively in HIV carriers, leading to cirrhosis and liver failure in a relatively short period [9]. A comprehensive population-based study on cirrhosis has been conducted in England [10]. Chronic HCV is frequently observed in individuals with HIV infection, hemophiliacs, transfusion recipients, and injecting drug users (IDUs) [11]. Multiple hepatitis virus co-infections, as opposed to a single hepatitis virus co-infection or HIV infection alone, are associated with an increased risk of severe liver abnormalities in HIV patients. Consequently,

immunosuppression plays a crucial role in the progression of liver disease [12]. Liver function tests (LFTs) are used to assess liver damage, function, or impairment [13]. Elevated liver enzyme levels are typically observed in cases of liver damage. The elevation of these enzymes in liver disease is attributed to their normal presence within hepatocytes. Factors such as excessive alcohol consumption, obesity (particularly in men), and smoking (in women) contribute to increased liver enzyme levels [14]. Therefore, alterations in LFTs indicate liver damage or the presence of liver disease. Although changes in LFT patterns aid in diagnosing liver disease, they do not specifically correlate with infection-induced liver disease [15]. In cases of hepatitis associated with HBV and HCV infection, LFTs play a pivotal role in diagnosis and in determining disease progression [16]. Alterations in liver enzyme patterns can result from various mechanisms; thus, it is essential to evaluate them individually. Hepatitis, co-infection, alcohol consumption, or concomitant medications are more likely to cause changes or alterations in liver enzymes. Hence, this study was planned to evaluate the liver enzymes of normal and HBV, HCV, HDV, and HIV patients.

MATERIALS AND METHODS

Study area and sample processing

A total of 1,012 serum samples were collected, comprising 512 cases of liver disease and 312 patients co-infected with an alcoholic condition. Among 500 healthy individuals, 70 exhibited an alcoholic condition, which was relatively infrequent within the study cohort. The samples were obtained at KAP Viswanathan Government Medical College and Annal Gandhi Memorial Government Hospital in Tiruchirappalli, Tamil Nadu, India, with appropriate patient consent and ethical approval (PR22AGKS015). All samples were processed at Mothercell Regenerative Centre, located on the 1st Floor, Anna Nagar, Tiruchirappalli, Tamil Nadu, India. The serum samples were handled under sterile and static conditions using proper techniques. After blood collection, the serum was separated from the clot to prevent hemolysis, and samples were stored in a refrigerator at 2 to 8°C for up to one week.

Serological analysis

In this serological investigation, third-generation ELISA kits were employed to screen serum samples for hepatitis and HIV markers. Hepatitis B surface antigen (HBsAg) was identified using Hepalisa (J. Mitra and Co. Pvt. Ltd., India), while the Hepatitis B envelope antigen (HBeAg) and its specific antibodies (anti-HBe) were detected with ELISA kits from Bio-Rad Laboratories, USA. Antibodies specific to HCV and HIV were determined using Microlisa kits (J. Mitra and Co. Pvt. Ltd., India).

Biochemical analysis

Hepatic function assessments, including albumin, bilirubin, and the enzymes serum alkaline phosphatase (SAP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase, were performed using commercial kits from Euro Diagnostics and Ecoline.

Statistical Analysis

Final data were interpreted by using the statistical software SPSS, version 13.0. P value of < 0.05 was considered statistically significant.

RESULT

In this study, clinical diagnoses were performed on 512 serum samples from patients with liver diseases (HBV, HCV, HDV, and HIV) and 500 samples from healthy individuals. The findings revealed that nearly all of the 29 HCV-positive cases exhibited significantly elevated bilirubin levels and enzyme activity, except for albumin (Table 1 and Suppl. Figure 1). Patients with chronic liver disease and elevated liver function test (LFT) levels showed a higher likelihood of developing cirrhosis. Serum alkaline phosphatase (SAP) levels were notably elevated in patients co-infected with HCV, HBV, and HIV, indicating liver damage, especially when SAP levels were double or triple those of controls. Similar to HCV and HBV co-infected patients, SAP levels were elevated in HIV and HCV-positive cases compared to other enzymes and pigments tested. In patients positive for HCV, HBV, and HIV co-infection, elevated levels of pigments and most enzymes, including total bilirubin, direct bilirubin, SAP, and gamma-glutamyl transferase (GGT), were observed, indicating abnormal liver function. High bilirubin levels in co-infected patients may suggest cirrhosis (Table 2 and Suppl. Figure 2). A liver function test using one-way analysis of variance revealed highly significant differences between normal and liver disease patients (Table 3). Duncan's post hoc analysis was conducted to compare the range of values between liver disease patients and controls. The total bilirubin values in different liver disease patients formed a single subset, indicating uniformity among them. The values of bilirubin, both direct and indirect, were categorized into five subsets, indicating variability among liver disease patients. Aspartate aminotransferase (AST) and albumin formed two subsets, whereas the values of SAP, alanine aminotransferase (ALT), and GGT in different liver disease patients formed a single subset. In the bilirubin direct and indirect tests, homogeneous subgroups were highly prevalent. Notably, individuals with normal ALT levels recover at a rate comparable to those with elevated ALT levels (Table 4 and Suppl. Figure 3). A Mann-Whitney test was conducted on randomly selected groups of HCV-positive patients and HCV-and-HBV-co-infected patients. Although bilirubin total, SAP, GGT, and albumin levels were elevated in HCV versus HCV/HBV co-infection, the difference was not statistically significant. However, bilirubin, ALT, and AST levels did show significant differences (Suppl. Table 1). In comparisons between HCV and HBV versus HCV and HIV co-infection, the LFT values were not

significant. In HCV and HBV versus HCV, HBV, and HIV co-infection, bilirubin direct, total, and ALT levels were significantly different, while others were not significant (Suppl. Table 2). Similarly, HCV and HBV-co-infected patients were compared with HCV, HBV, and HIV-co-infected patients (Suppl. Table 3). In HCV and HIV versus HCV, HBV, and HIV co-infection, only the difference in ALT was significant, while other comparisons were not significant (Suppl. Table 4).

The findings reveal that in nearly all coinfected patients, most of the enzymes and pigments assessed were elevated. Among HCV-positive patients who regularly consume alcohol, liver enzyme and pigment levels were increased, though these elevations did not reach statistical significance (Table 5). Similarly, in patients coinfected with HCV and HBV who also consumed alcohol, liver enzymes and pigments were elevated, yet the increase was not statistically significant (Table 6). When comparing HCV and HIV coinfected patients who were non-alcoholic to those with a drinking habit, the latter group exhibited elevated liver function test (LFT) results (Table 7). Comparable observations were made in individuals coinfected with HCV, HBV, and HIV who consumed alcohol (Table 8).

DISCUSSION

The findings illuminate the complex relationship between viral infections and liver function, emphasizing a notable increase in liver enzymes among HCV-positive individuals, especially those with co-infections or alcohol consumption. Previous research has shown that individuals displaying signs or symptoms of chronic liver disease [17] are more likely to have a history of chronic HBV infection [18]. A significant portion of patients with chronic liver disease and elevated liver function tests (LFTs) may eventually develop cirrhosis. In this study, both chronic liver disease cases with and without cirrhosis showed high LFT levels. However, after a decade of follow-up, patients with consistently normal alanine aminotransferase (ALT) levels progressed to cirrhosis at a slower pace [19]. Among patients co-infected with HCV and HBV, serum alkaline phosphatase (SAP) levels were significantly higher compared to other enzymes and pigments. Elevated SAP levels indicate hepatic damage. Furthermore, when SAP levels are two to three times higher than control levels, they are often considered mildly elevated. Elevated levels of SAP, ALT, and bilirubin suggest liver damage, cirrhosis, or viral replication, supporting previous studies that highlight the impact of these infections on liver health. The prevalence rate of HCV (5.6%) in this study surpasses global averages, potentially reflecting regional variations or co-factors such as alcohol use that worsen liver damage. A one-way ANOVA revealed significant variance between liver disease patients and healthy individuals. Duncan's post hoc analysis indicated that total bilirubin levels did not significantly differ among liver disease patients, while other parameters such as aspartate aminotransferase (AST) and albumin formed distinct subsets. The Mann-Whitney test demonstrated significant differences in ALT, bilirubin, and AST levels between HCV and HCV/HBV co-infected patients. Comparisons of HCV versus HCV and HIV co-infection, as well as HCV/HBV/HIV co-infection, also revealed significant differences in certain LFT parameters, particularly ALT. The LFT conducted using a one-way analysis of variance showed highly significant variance between normal and liver disease patients, as presented in Table 3. Duncan's post hoc analysis was conducted to compare the range of values between liver disease patients and controls. The total bilirubin values in different liver disease patients formed a single subset, indicating uniformity in the results among them.

Infection with HBV and HCV is widespread in regions where these viruses are endemic [20]. HCV is the most prevalent chronic RNA virus affecting humans, with around 170 million people infected worldwide [21]. Chronic liver disease caused by the Hepatitis group of viruses includes chronic hepatitis (both active and inactive), cirrhosis, and hepatocellular carcinoma (HCC) [22]. Globally, over 500,000 new cases of HCC are reported each year [13,23]. In the United States, where the risk from aflatoxin is low, HCV infection accounts for one-third of all HCC cases [24]. However, mass vaccination against HBV has significantly reduced incidence and mortality. Elevated liver function tests (LFT), particularly alanine aminotransferase (ALT), are notably higher in patients with viral co-infections compared to those with a single infection. Various studies have shown that elevated ALT levels indicate viral replication [25]. Individuals positive for both HBV and HDV exhibited decreased ALT levels [26,27]. In contrast, nearly all patients in our study infected with one or more viruses showed elevated ALT levels, attributed to liver cells serving as sites of viral infection and proliferation due to their virulence and liver tropism. Patients with normal ALT levels exhibit considerably less inflammation. In this study, however, ALT levels increased in most individuals with chronic liver disease. Alghamdi et al. (2016) [28] found that the mean ALT value in co-infected patients was higher than in the HIV-negative group, although the difference was not statistically significant. Thus, this study clearly illustrates the direct correlation between LFT and bloodborne viruses. In the case of hepatitis C virus infection, the prevalence rate is 5.6%, exceeding the global HCV prevalence, which ranges from 0.2% to 2%. There is a relatively low prevalence of HCV antibodies among blood donors in the US and Northern Europe, including the UK, France, and Germany [29-31]. Alcohol consumption is a well-established cause of liver disease, particularly chronic hepatitis [32]. The LFT profiles of alcoholic and non-alcoholic HCV-positive patients in this study with chronic hepatitis showed elevated levels of liver enzymes. In alcoholic and non-alcoholic HCV and HBV co-infected patients, serum alkaline phosphatase (SAP) and gamma-glutamyl transferase (GGT) were significantly elevated compared to other enzymes and pigments [33]. Alcohol generally worsens liver function, which, combined with viral infection, aggravates the condition. Elevated LFT was observed in 74 HIV patients in a study by Shamana et al. (2016) [34, 35]. Prospectively followed HIV-positive patients were referred for abnormal LFT tests, with 3.8% having dual or triple hepatitis co-infection, and 69.5% being HBV or HCV mono-infected.

The current study's findings on liver function tests reveal abnormal levels of liver pigments, including total, direct, and indirect bilirubin, alongside sudden increases in liver enzymes such as SAP, ALT, AST, GGT, and albumin in patients

with liver diseases. The elevated liver function test (LFT) levels observed in patients with chronic hepatitis, especially those who consume alcohol, underscore the aggravating effects of alcohol and viral infections. These results align with other studies linking high LFT levels to viral replication and the progression of liver disease. In HIV patients, elevated liver enzymes were noted, potentially due to multiple factors, including co-infections and antiretroviral therapy, complicating the clinical management of liver disease in these populations. Additionally, this study identified elevated liver enzyme levels in HCV-positive patients. HIV patients often show higher liver enzyme levels, which may stem from various factors such as concurrent HAART and alcohol consumption, opportunistic infections, steatohepatitis, or multiple viral hepatitis infections. This study also highlights the need for comprehensive monitoring of liver function in patients with viral hepatitis and emphasizes the importance of managing co-infections and alcohol use to mitigate liver damage.

CONCLUSION

Serum samples were collected from 1,012 individuals, including 512 diagnosed with liver disease and 312 who are co-infected and regularly consume alcohol. Interestingly, 70% of a control group of 500 healthy, non-drinking individuals were identified as alcoholics. The findings of this study are consistent with previous research, which has shown elevated liver enzyme levels in individuals infected with hepatitis viruses. A plausible explanation for this is the significant alteration or impairment of liver function in infected individuals compared to healthy controls. Although elevated serum alkaline phosphatase (SAP) levels were observed in only a small fraction of cases, they were consistently elevated in all cases of cirrhosis and in the patient positive for hepatitis C virus (HCV). The study further identified that infection with hepatitis B virus (HBV), rather than other viral infections, was responsible for the observed severity.

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CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among the authors.

ETHICAL APPROVAL

Applicable

DATA AVAILABILITY STATEMENT

All the raw data available with corresponding author, the data will send on mail request.

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List of Tables

Table 1. HCV positive and HCV and HBV Coinfected patients of LFT.

Category	Liver Function test							
	Bilirubin (mg%)			Serum ALP (KA units)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Albumin (gm %)
	Total	Direct	Indirect					
Control	0.5240 ±0.226	0.220 ± 0.055	0.396 ± 0.155	72.127 ± 21.563	36.56 ± 09.35	23.87 ± 07.55	70.60 ± 12.03	2.74 ± 0.116
HCV +ve patients	3.411± 0.882	0.633 ± 0.1826	0.935 ± 0.018	143.780 ± 13.826	62.159 ± 06.705	48.462 ± 05.527	98.596 ± 09.575	3.97 ±0.24
p-value	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000
Control	0.56 ± 0.17	0.217 ±0.084	0.486 ± 0.164	68.825 ± 06.665	32.175 ±11.094	29.287 ± 04.520	64.437 ± 14.744	2.63 ±0.25
HCV and HBV co-infect	3.63 ± 0.96	1.58 ± 0.79	1.637 ± 0.523	141.712 ±11.942	57.8 ± 03.23	39.422 ± 01.018	99.352 ± 07.190	3.15 ± 0.26
p-value	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. HIV, HBV, and HCV Coinfected patients with different sample sizes and LFT.

Samples	Category	Liver Function Test							
		Bilirubin (mg%)			ALT (IU/L)	Serum ALP (KA units)	AST (IU/L)	GGT (IU/L)	Albumin (gm/%)
		Total	Direct	Indirect					
HCV and HIV coinfected patients	Control	0.618 ± 0.128	0.212 ± 0.067	0.473 ± 0.139	30.48 ± 11.46	67.0 ± 6.954	30.51 ± 4.52	62.25 ± 11.23	2.31 ± 0.42
	Sample	2.810 ± 1.22	1.963 ± 0.932	1.987 ± 7.72	56.78 ± 4.401	135.62 ± 11.275	38.36 ± 3.17	98.10 ± 5.74	3.03 ± 0.77
	p-value	0.000	0.001	0.001	0.001	0.000	0.001	0.000	0.000
HCV, HBV, and HIV coinfected patients	Control	0.572 ± 0.075	0.265 ± 0.110	0.357 ± 0.128	32.87 ± 14.53	69.89 ± 4.326	33.77 ± 3.87	65.71 ± 13.741	1.93 ± 0.87
	Sample	3.397 ± 1.272	3.232 ± 0.478	0.272 ± 0.543	65.27 ± 3.97	140 ± 18.254	41.71 ± 3.17	99.92 ± 3.06	3.24 ± 0.01
	p-value	0.009	0.000	0.000	0.005	0.000	0.006	0.003	0.004

Table 3. One-way analysis of variance between normal and liver disease patients

Liver function test	Variance	Sum of squares	df	Mean square	F	Sig

Bilirubin Total	Between groups	151.503	4	37.876	66.212	0.000
	Within groups	40.615	71	0.572		
Bilirubin Direct	Between groups	46.897	4	11.724	86.723	0.000
	Within groups	0 9.463	71	0.135		
Bilirubin indirect	Between groups	31.202	4	7.800	84.615	0.000
	Within groups	6.545	71	9.210		
Serum alkaline phosphatase	Between groups	86348.983	4	21587.246	76.233	0.000
	Within groups	20105.406	71	283.175		
Alanine aminotransferase	Between groups	9824.843	4	2456.211	44.420	0.000
	Within groups	3925.916	71	55.295		
Aspartate aminotransferase	Between groups	8388.072	4	2097.018	57.848	0.000
	Within groups	2573.767	71	36.250		
Gamma-glutamyl transpeptidase	Between groups	15126.712	4	3746.121	42.127	0.000
	Within groups	6367.704	71	91.117		
Albumin	Between groups	33.348	4	8.313	24.226	0.000

Table 4. LFT liver diseased patient and viral infection in Duncan test.

LFT	**Viral infection**	**Sample size**	**Subset**				
			1	**2**	**3**	**4**	**5**
Bilirubin total (mg%)	Control	29	0.5241				
	HCV and HIV	6		2.8910			
	HCV, HBV and HIV	4		3.1580			
	HCV	29		3.3124			
	HCV and HBV	8		3.6974			
	Sig	0	1.0000	0.0589			
Bilirubin direct (mg%)	Control	29	0.2179				
	HCV	29		0.6231			
	HCV and HBV	8			1.492		
	HCV and HIV	6				1.9850	
	HCV, HBV and HIV	4					3.1082
	Sig		1.0000	1.0000	1.0000	1.0000	1.0000
Bilirubin indirect (mg%)	Control	29	0.3485				
	HCV	29		0.9459			
	HCV and HBV	8			1.6233		
	HCV and HIV	6			2.6210	1.9867	
	HCV, HBV and HIV	4					2.6450
	Sig		1.0000	1.0000	1.0000	1.0000	1.0000
	Control	29	73.227				

Serum alkaline phosphatase (KA units)	HCV and HIV	6		133.123			
	HCV, HBV and HIV	4		140.120			
	HCV and HBV	8		140.742			
	HCV	29		143.545			
	Sig		1.0000	0.2570			
Alanine aminotransferase (IU/L)	Control	29	36.4555				
	HCV and HIV	6		55.9871			
	HCV, HBV and HIV	4		65.3270			
	HCV	29		62.1924			
	HCV and HBV	8		58.0000			
	Sig		1.0000	0.0590			
Aspartate aminotransferase (IU/L)	Control	29	24.7793				
	HCV and HIV	6		38.9750			
	HCV, HBV and HIV	4		42.7950			
	HCV	29		48.3711			
	HCV and HBV	8		40.3834			
	Sig		1.0000	0.3220	0.0650		
Gamma glutamyl transpeptidase (IU/L)	Control	29	69.7034				
	HCV and HIV	6		96.9750			
	HCV, HBV and HIV	4		99.8760			
	HCV and HBV	8		99.4712			
	HCV	29		98.3471			
	Sig		1.0000	0.643			
Albumin (% in gms)	Control	29	1.5979				
	HCV and HBV	8		2.7256			
	HCV, HBV and HIV	4		2.8950	2.8650		
	HCV	29		2.7175			
	HCV and HIV	6			3.5600		
	Sig		1.0000	0.6223	0.1040		

Table 5. Liver Function Test in Alcoholic and Non-alcoholic HCV-infected Patients

**The values are mean ± S.D of a sample size of 4*

Category	**Liver Function Test**							
	Bilirubin (mg%)			**Serum ALP (KA units)**	**ALT (IU/L)**	**AST (IU/L)**	**GGT (IU/L)**	**Albumin (gm%)**
Non-alcoholic with HCV and HBV coinfection	2.087 ± 0.873	2.00 ±0.307	2.33 ± 1.31	82.88 ± 8.763	38.72 ± 4.42	35.65 ± 3.838	80.92± 13.78	3.160 ± 0.681
Alcoholic with HCV and HBV coinfection	3.115 ± 1.350	4.51 ± 1.94	4.530 ± 1.11	152.15 ± 22.34	62.90 ± 4.60	45.05 ± 7.455	112.10± 8.30	3.210 ± 0.577

p-value	0.249	0.043	0.044	0.001	0.000	0.066	0.008	0.915

Table 6. Liver Function Test in Alcoholic and Non-alcoholic HCV and HBV coinfected patient

Category	Liver Function Test							
	Bilirubin (mg%)			Serum ALP (KA units)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Albumin (gm%)
	Total	Direct	Indirect					
Non-alcoholic with HCV	1.5150 ± 0.526	1.587 ± 0.681	2.471 ± 1.441	80.060 ± 14.06	41.650 ± 10.57	35.40 ± 5.858	85.175 ± 15.663	2.78 ± 0.462
Alcoholic with HCV	4.465 ± 2.409	4.314 ± 1.315	4.517 ± 1.84	141 ± 17.301	71.850 ± 5.105	48.55 ± 7.813	110.97± 7.71	3.125 ± 0.078
p-value	0.054	0.017	0.137	0.011	0.002	0.036	0.025	0.083

**The values are mean ± S.D of a sample size of 4*

Table 7. Liver Function Test in Alcoholic and Non-alcoholic HCV and HIV coinfected Patients

Category	Liver Function Test							
	Bilirubin (mg%)			Serum ALP (KA units)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Albumin (gm%)
	Total	Direct	Indirect					
Non-alcoholic with HCV and HIV coinfection	1.915	1.105	1.500	61.050	41.45	36.75	77.90	2.20
Alcoholic with HCV and HIV coinfection	2.430	2.855	2.140	157.95	62.30	40.35	111.50	2.265

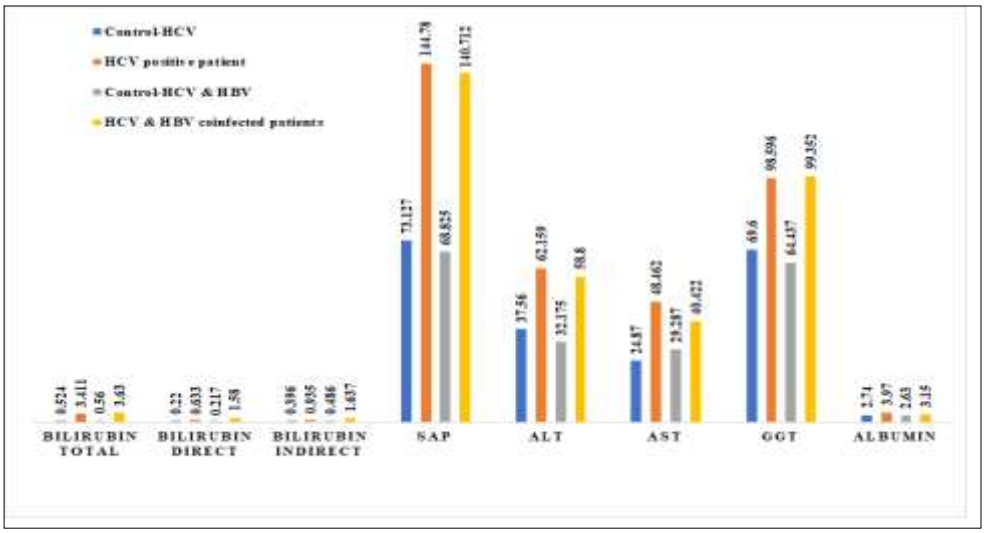
**The values are the mean of two estimations. Since the sample size was only 2 the 't' test was not performed.*

Table 8. Liver Function Test in Alcoholic and Non-alcoholic HCV, HBV, and HIV coinfected patients

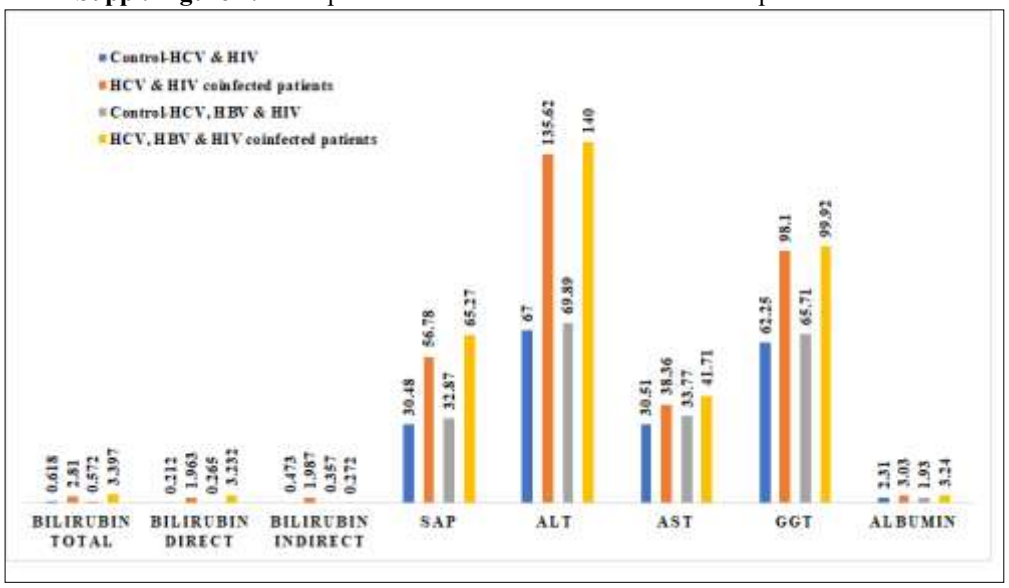
Category	Liver Function Test							
	Bilirubin (mg%)			Serum ALP (KA units)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Albumin (gm%)
	Total	Direct	Indirect					
Non-alcoholic with HCV, HBV and HIV coinfection	1.830	1.310	1.270	62.50	44.01	37.58	100. 46	1.845
Alcoholic with HCV, HBV and HIV coinfection	2.40	2.205	2.205	140.85	65.40	41.20	110.67	2.115

** The values are the mean of two estimations. Since the sample size was only 2 the 't' test was not performed*

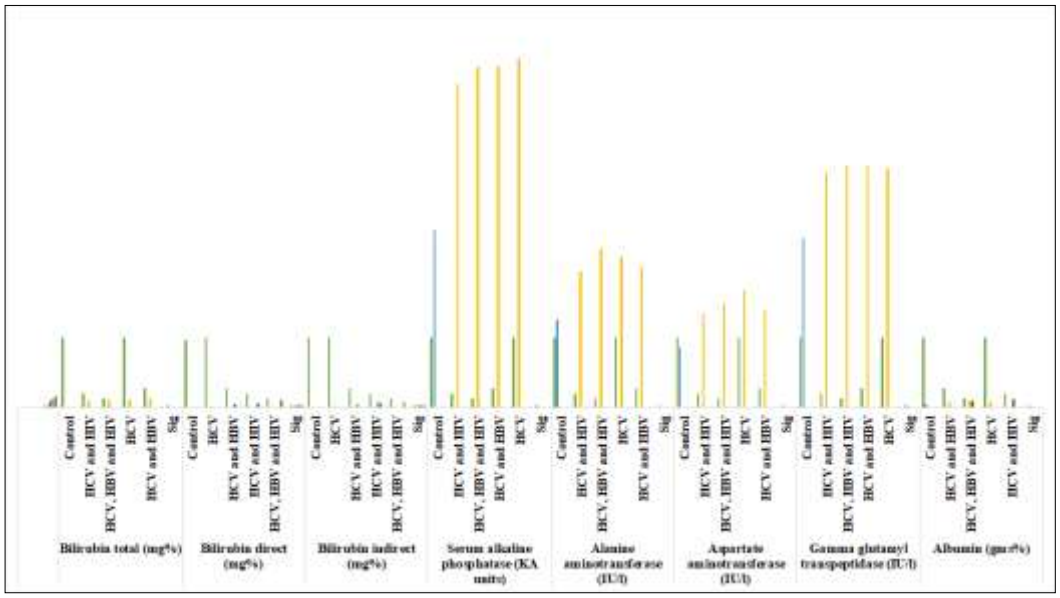
Supplementary Files
Manuscript: "Comparative Study on Liver Function Tests in HBV, HCV, HIV Infected and Co-infected Patients with Alcoholic Habit



Suppl. Figure 1. HCV positive and HCV and HBV Coinfected patients of LFT.



Suppl. Figure 2. HIV, HBV, and HCV coinfected patients with different sample sizes and LFT.



Suppl. Figure 3. Viral infection and LFT of liver disease patients in Duncan test

Suppl. Table 1. Liver Function Test by Mann-Whitney - Table of ranks

Liver function test	Viral Infection (Mean Rank)

	HCV	HCV & HBV	HCV	HCV & HIV
Bilirubin total	18.02	20.55	18.23	14.40
Bilirubin direct	15.42	32.32	14.34	31.42
Bilirubin indirect	15.54	31.21	16.22	30.74
Serum alkaline phosphatase	18.91	15.94	18.90	11.54
Alanine aminotransferase	21.98	13.49	18.89	10.78
Aspartate aminotransferase	23.61	06.90	21.52	06.71
Gamma glutamyl transpeptidase	17.46	21.97	16.94	19.42
Albumin	17.89	21.49	15.97	24.32

**Sample size: HCV-29 and HCV & HBV- 8: HCV-29 and HCV & HIV - 6*

Suppl. Table 1a. Liver Function Test by Mann-Whitney test

Test	Bilirubin total (mg%)	Bilirubin direct (mg%)	Bilirubin indirect (mg%)	ALT (IU/L)	Serum ALP (KA units)	AST (IUL/L)	GGT (IU/L)	Albumin (gm%)
HCV Vs HCV and HBV								
Mann-Whitney U	103.000	11.100	19.450	94.500	62.400	21.100	92.564	105.400
Sig (2 tailed)	0.645	0.0	0.0	0.477	0.033	0.0	0.383	0.741
. HCV Vs HCV and HIV								
Mann-Whitney U	56.500	7.100	3.600	45.700	37.960	12.750	84.780	41.980
Sig (2 tailed)	0.202	0.0	0.0	0.076	0.031	0.0	0.881	0.050

Suppl. Table 2. Liver Function Test – Mann-Whitney test - Table of rank

Liver function test	Viral Infection			
	HCV	HCV, HBV and HIV	HCV & HBV	HCV & HIV
Bilirubin total	16.96	13.29	8.26	6.18
Bilirubin direct	14.93	31.42	6.59	8.41
Bilirubin indirect	14.10	30.86	6.21	9.32
Serum alkaline phosphatase	16.58	12.50	7.91	5.54
Alanine aminotransferase	15.47	21.00	7.84	7.12
Aspartate aminotransferase	17.16	6.32	8.20	5.93
Gamma glutamyl transpeptidase	15.97	20.72	9.58	7.43
Albumin	15.78	18.08	6.98	10.47

**Sample size: HCV-29 and HCV, HBV & HIV-4: HCV & HBV-8 and HCV & HIV – 6*

Suppl. Table 2a. Liver Function Test - Mann-Whitney test

Test	Bilirubin total	Bilirubin direct	Bilirubin indirect	Serum ALP	ALT	AST	GGT	Albumin
HCV Vs HCV, HBV and HIV								
Mann-Whitney U	46.900	0.000	0.000	43.400	37.500	16.700	39.500	55.500
Sig (2 tailed)	0.556	0.0	0.0	0.462	0.315	0.028	0.418	0.742
HCV & HBV Vs HCV & HIV								
Mann-Whitney U	14.900	17.600	13.300	13.600	21.500	20.000	18.500	12.100
Sig (2 tailed)	0.271	0.424	0.171	0.171	0.622	0.682	0.319	0.118

Suppl. Table 3. LFT- Mann-Whitney test – Rank table

Liver function test	Viral Infection			
	HCV & HBV	HCV, HBV and HIV	HCV & HIV	HCV, HBV & HIV
Bilirubin total	7.26	5.48	6.43	6.52
Bilirubin direct	4.60	10.40	5.10	8.65
Bilirubin indirect	4.45	10.10	5.40	6.10
Serum alkaline phosphatase	7.94	6.90	6.10	7.35
Alanine aminotransferase	5.91	10.86	4.74	9.14
Aspartate aminotransferase	6.40	9.30	5.21	8.65
Gamma glutamyl transpeptidase	7.24	7.43	6.37	6.97
Albumin	7.91	6.72	7.87	4.94

Suppl. Table 4. Liver Function Test by Mann-Whitney test

Test	Bil-i	Bil-ir	Bil-ir	Serum	ALT	AST	GGT	Alb
HCV and HBV Vs HCV, HBV and HIV								
Mann-Whitney U	11.600	0.000	2.100	14.000	2.600	8.200	15.400	13.600
Sig (2 tailed)	0.445	0.006	0.026	0.871	0.014	0.324	0.973	0.692
HCV & HIV Vs HCV, HBV & HIV								
Mann-Whitney U	11.000	3.000	6.000	9.000	1.000	3.000	10.500	5.500
Sig (2 tailed)	0.914	0.067	0.257	0.610	0.019	0.067	0.762	0.171