



Original Article

Comparison of Biochemical Parameters in Patients with Hepatitis B, C, and Dual Hepatitis B and C in Northwest Pakistan

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Abstract

The leading causes of hepatitis are viral infections, Hepatitis B virus (HBV) and Hepatitis C virus (HCV). Millions of people have been infected with these deadly viral infections worldwide, and in Pakistan, every tenth person is infected with these viruses. Different populations respond with different rates to infectious diseases due to host genomic differences. To evaluate and compare the biochemical parameters in different types of hepatitis (Hepatitis B, C, and Co-infection) and different ethnic groups, a total of 200 pre-screened patients were recruited from District Headquarters Teaching Hospital Dera Ismail Khan and Tank. Blood samples (5ml) were taken from patients and were assayed for biochemical parameters, including four liver function tests (LFTs) and two renal function tests (RFTs). In 200 patients, the mean scores of Alanine transaminase (ALT) were 376 ± 335 , 315 ± 265 , and 478 ± 519 IU/L in HBV, HCV, and co-infected patients, respectively. Moreover, the mean score of ALT was 31 ± 7.2 IU/l in the normal control group. All other biochemical parameters demonstrated elevated levels in co-infection, HBV, and HCV, respectively, except total proteins. The RFTs showed a threshold or upper normal limit (UNL); nonetheless, when compared to normal control subjects, RFTs parameters were high in infected patients, as compared to normal control. Ethnicity wise comparison of parameters indicated that Pushtoon ethnic group indicated a high degree of severity of HBV infection and co-infection, as compared to Saraiki and Rajpoot ethnic groups, while Saraiki ethnic group showed a higher severity of HCV than both of Pushtoon and Rajpoot. Rajpoot ethnic group was least affected than both Pushtoon and Saraiki ethnic groups. Co-infected patients were more severely affected, as compared to HBV and HCV patients. The ethnicity-wise study provided evidence that different ethnic groups showed different degrees of severity. There may be some genetic background involved in hepatitis B and C viral infection due to which all three ethnic groups showed different degrees of severity. In gender-wise comparisons, male patients were more affected than female patients.

Keywords: Co-infected, HBV, HCV, LFT, RFT

1. Introduction

In Pakistan, hepatitis viruses are endemic in nature. There is a high burden of hepatitis A to E, with maximum morbidity in hepatitis A and E, as well as maximum mortality in hepatitis B, C, and D. Hepatitis may be acute (short term) or chronic (long term).

Hepatitis infection lasting more than six months is referred to as chronic hepatitis. Hepatitis A and E are transmitted by polluted water and food. Blood-borne viruses, such as Hepatitis B virus (HBV) and hepatitis C virus (HCV), may only be transmitted via contact with infected body fluids (1). The mode of spread and sequel

of HBV and HCV are almost similar. The leading causes of hepatitis are viruses across the globe (2).

Serious health issues, such as bleeding disorders, the buildup of fluid in the abdomen (ascites), memory loss, and fatigue due to the buildup of toxins, namely ammonia, occur in chronic Hepatitis B and C viral infections. Kidneys failure, decompensated liver, cirrhosis, and hepatocellular carcinoma are other more complicated and fatal problems that occur in chronic hepatitis (3, 4). Co-infection is common since both viruses are parentally transmitted. In case of co-infection, HCV infection is preferably first treated since the HCV virus is dominant over HBV. Another drawback is that when HCV is cleared, HBV attacks the liver more aggressively. Co-infected patients are at a higher risk of developing cirrhosis and liver cancer, as compared to mono-infected ones (5).

The HBV and HCV infections contribute to the global public health threats in most developing countries, where health care systems lack the safety measures necessary to avert the risks of infection and public awareness about the modes of transmission is insufficient (6, 7). According to a survey study conducted by the regional office of the world health organization for South-East Asia, it was reported that countries with the highest chronic infection of HBV and HCV are Egypt (22%), Pakistan (4.8%), and China (3.2%). In these countries, the cause of viral spread may be associated with the use of contaminated items, such as needles, razors, acupuncture needles, hemodialysis, needle stick injury, and tattooing equipment (8).

A survey conducted by Pakistan Medical Research Council (PMRC) revealed that in Pakistan, around 7.4% of the population are suffering from HBV (2.4%) and HCV (4.9%) (9). Therefore, Hepatitis B and C are highly endemic in Pakistan. The reason may be the lack of proper health facilities, poor economic status, and poor public awareness about the transmission of major communicable diseases, including HBV, HCV, and HIV (10). Studies are too limited to give a clear picture

of the prevalence of HBV and HCV at the national level, especially among otherwise healthy individuals.

The majority of previous studies targeted different small groups of individuals with some clinical indications; therefore, they do not accurately reflect the overall prevalence in Pakistan (11, 12). Dera Ismail Khan is the 5th largest populated city in the North West of Pakistan. Its population is increasing at a faster rate due to the migration of masses from different small towns, villages, as well as Afghan refugees who were settled in Waziristan. Major ethnic groups in this area are Pushtoons, Saraiki, and Rajpoot. In light of the aforementioned issues, the present study aimed to evaluate and compare the biochemical parameters in different types of hepatitis (Hepatitis B, C, and Co-infection) and different ethnic groups.

2. Materials and Methods

2.1. Study Subjects

Pre-screened (200) Hepatitis B, C, and co-infected patients from both genders were included in this study. The purpose was to compare the severity among different groups; therefore, patients with Alanine transaminase (ALT) >90 IU/l were excluded. The research was performed according to the provisions of the World Medical Association and Declaration of Helsinki (as revised in Edinburgh 2000).

2.2. Blood Sample Collection and Centrifugation

Blood samples were collected from 200 prescreened Hepatitis B, C, and Co-infected patients of different ethnic groups (Pashtuns, Siraiki, and Rajpoot) admitted to District Head Quarter (DHQ) Teaching Hospital and Mufti Mahmoud Memorial Teaching (MMMT) Hospital Dera Ismail Khan by the help of disposable syringe from 1st January 2019 to 31st December 2019. Three ml of blood was transferred for biochemical parameters and centrifuged at 1000 rpm for 15 min for the separation of plasma.

2.3. Liver Function Tests

2.3.1. Alanine Transaminase Determination

The kinetic method was used for ALT determination

as described by Schumann, Bonora (13).

2.3.2. Alkaline Phosphatase Determination

Alkaline phosphatase (ALP) was measured by the Alkaline Phosphatase kit. A standard method was used as described by the German Society of Clinical chemistry (GSCC).

2.3.3. Bilirubin Determination

Bilirubin was determined using Jendrassik-Grof, (France) kit by the standardized method of Koc, Robaey (14).

2.3.4. Total Protein Determination

For total protein determination total Protein Biuret Human Diagnosis worldwide kit was used. A standardized method was used as published by Gornall, Bardawill (15).

2.4. Renal Function Test

2.4.1. Creatinine Determination

For Creatinine determination, the Creatinine Jaffe kit was used Vasiliades (16).

2.4.2. Blood Urea Determination

Blood urea was determined by the Urea UV diagnoses kit as described by Taylor and Vadgama (17).

2.5. Statistical Analysis

The descriptive and inferential analyses were performed in SPSS software (version 20), released by IBM USA. The data were observed on two scales (ordinal and ratio scales). The age of the subjects was initially measured on ratio scales. Nonetheless, for association analysis and to observe the independence of different attributes, age was transformed into an ordinal measurement scale. Different statistical tools included student t-test to compare active and controls groups. The tests were performed at a 1% level of significance, and statistical significance in different quantitative and qualitative aspects of the study was observed. Descriptions were made considering normal bands that were developed on the basis of average and variation of study phenomena.

3. Results

For the comparison of biochemical parameters in Hepatitis B, C, and co-infected (HBV+HCV) patients,

200 patients were enrolled from three ethnic groups (Pushtoon, Saraki, and Rajpoot) and normal control. The comparison of biochemical parameters was made among three ethnic groups. A total of 60 patients were enrolled in each hepatitis type, including Pushtoon (n=20), Saraki (n=20), and Rajpoot (n=20). A number of 20 normal control were also enrolled as displayed in table 1. The patients were within the age range of 28-65 years. Both male and female patients were enrolled.

Biochemical parameters consisted of liver function tests (LFTs) and renal/kidneys function tests (RFTs). The LFTs comprised Alanine aminotransferase (ALT) and alkaline phosphates (ALP) tests, while RFTs included blood urea and creatinine tests. Higher levels of LFTs, especially ALT (as demonstrated in tables 2-4 and 5), and RFTs were noted in co-infected patients, as compared to mono-infected ones. Nonetheless, among single infection (HBV/HCV), HBV demonstrated a huge rise in LFTs, except for total protein which showed a decreased level in many severely infected patients (minimum up to 3.1 g/dl than the normal range which is 6-8 g/dl). While in some patients, it was at a lower level of normal (LLN) 6 g/dl, when compared to the mean of control subjects which was 6.8 g/dl. In RFTs, the mean blood urea and creatinine were around the Upper limits of normal (ULN) which was 40-50mg/dl. Nevertheless, some more severe hepatitis patients indicated a rise in RFTs, blood urea > three times ULN (150 g/dl), and serum creatinine > four times ULN (5.2 mg/dl), while some patients showed threshold or ULN (1-1.5mg/dl). The gender-wise study indicated that male patients exhibited a dramatic rise in LFTs and RFTs, as compared to female patients.

Table 1. Patient distribution according to ethnicity and hepatitis type

Ethnicity	HBV	HCV	Co-infection	Normal
Pushtoon	n=20	n=20	n=20	
Saraiki	n=20	n=20	n=20	n=20
Rajpoot	n=20	n=20	n=20	

Table 2. Liver function tests of Hepatitis B infected patients

Parameters	Ethnic groups with HBV infection						Control group		Normal range
	Pushtoon HBV		Saraiki HBV		Rajpoot HBV		Mean	SD	
LFTs	Mean	SD	Mean	SD	Mean	SD			Mean
ALT	415	309	390	428	324	258	31	7.2	10-49/1
ALP	308	111	291	84	275	92	164	46	70-305 U/l
Bilirubin	2.2	0.87	2.2	1.5	2.1	1.4	0.11	0.13	0.1-1.3 mg/dl
Total protein	5.8	1.7	6.1	1.5	6.5	1.1	6.8	2	6-8 g/dl

LFTs: liver function tests, ALT: Alanine transaminase, ALP: alkaline phosphates

Table 3. Liver function tests of Hepatitis C infected patients

Parameters	Ethnic groups with HCV infection						Control group		Normal range
	Pushtoon HCV infected		Saraiki HCV infected		Rajpoot HCV infected		Mean	SD	
LFTs	Mean	SD	Mean	SD	Mean	SD			Mean
ALT	324	283	355	273	265	243	31	7.2	10-49/ IU/l
ALP	273	120	305	106	301	109	164	46	70-305 IU/l
Bilirubin	2	1.2	2	1.3	1.9	1.2	0.11	0.13	0.1-1.3 mg/dl
Total. Protien	5.9	1.2	5.5	1.5	6.1	1.4	6.8	2	6-8 g/dl

LFTs: liver function tests, ALT: Alanine transaminase, ALP: alkaline phosphates

Table 4. Liver function tests of HBV-HCV co-infected patients

Parameters	Ethnic groups with Co-infection								Normal range
	Pushtoon ethnic		Saraiki ethnic		Rajpoot ethnic		Control group		
LFTs	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
ALT	518	575	464	579	451	410	31	7.2	10-49 IU/l
ALP	354	104	374	99	345	105	164	46	70-305 IU/l
Bilirubin	3.2	1.3	2.9	2.2	2.1	1.2	0.11	0.13	0.1-1.3 mg/dl
Total protein	4.9	1.6	5.9	1.1	5.7	1.3	6.8	2	6-8 g/dl

LFTs: liver function tests, ALT: Alanine transaminase, ALP: alkaline phosphates

Table 5. Biochemical parameters in HBV, HCV, and co-infected patients

Parameters	HBV infected (n=60)		HCV infected (n=60)		Co-infected (n=60)		Control group		Normal range
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	
Biochemical parameters									
ALT	376	335	315	265.47	478.08	519.4	31.6	7.21	10-49 U/l
ALP	292.18	96.12	293.63	111.48	358.18	102.5	164.4	46.05	70-305 U/l
Bilirubin	2.57	1.55	2.16	1.49	2.85	1.79	0.76	0.13	0.1-1.3 mg/dl
Total protein	6.01	1.54	5.84	1.41	5.54	1.43	6.82	2.03	6-8 g/dl
UREA	41.43	19.24	39.21	19.99	48.25	21.61	24.5	6.71	10-50mg/dl
Creatinine	1.47	0.72	1.4	0.83	1.68	1.11	0.73	0.12	0.1-1.5 mg/dl

LFTs: liver function tests, ALT: Alanine transaminase, ALP: alkaline phosphates

3.1. Descriptive Analysis

3.1.1. Alanine Aminotransferase in Hepatitis Type

In 200 patients, the mean scores of ALT were obtained at 376 ± 335 IU/L, 315 ± 265 IU/L, and 478 ± 519 IU/L in HBV, HCV, and co-infected patients, respectively. While in the normal control group, the mean score of ALT was 31 ± 7.2 IU/l as presented in tables 2- 4.

3.1.2. Effect of Ethnicity on Alanine Aminotransferase Level in Various Types of Hepatitis

The mean scores of ALT in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 415 ± 309 , 390 ± 428 , and 324 ± 258 IU/l, respectively, as illustrated in table 4. The mean scores of ALT in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were reported as 324 ± 283 , 355 ± 273 , and 265 ± 243 IU/l as displayed in table 4. The mean scores of ALT in Pushtoon, Saraiki, and Rajpoot co-infected patients were obtained at 518 ± 575 , 464 ± 579 , and 451 ± 410 IU/l, respectively, as shown in table 4. While in the normal control group, the mean score of ALT was 31 ± 7.2 IU/l as depicted in tables 2- 4.

3.1.3 Alkaline Phosphatase Level in Different Types of Hepatitis

The Alkaline phosphatase (ALP) levels in HBV, HCV, and co-infected patients were 292.45 ± 96.12 , 293.63 ± 111.48 , and 358 ± 102.52 IU/l, respectively. In the normal control group, the mean score of ALP was 164 ± 46 IU/l (Table 5).

3.1.4 Effect of Ethnicity on Alkaline Phosphatase Level in Various Types of Hepatitis

The mean scores of ALP in Pushtoon, Saraiki, and Rajpoot ethnic groups with HBV infection were 308 ± 111 , 291 ± 84 , and 275 ± 92 IU/l, respectively, as depicted in table 4. The mean scores of ALP in Pushtoon, Saraiki, and Rajpoot ethnic groups with HCV infection were 273 ± 128 , 305 ± 106 , and 301 ± 109 IU/l, respectively, as illustrated in table 3. The mean scores of ALP in Pushtoon, Saraiki, and Rajpoot co-

infected patients were obtained at 354 ± 104 , 374 ± 99 , and 345 ± 105 IU/l, respectively, as illustrated in table 4. While in the normal control group, the mean score of ALP was 164 ± 46 IU/l as displayed in tables 2, 3, and 4.

3.1.5 Comparison of Bilirubin among Different Types of Hepatitis

The mean scores of bilirubin were 2.57 ± 1.3 , 2.16 ± 1.2 , and 2.85 ± 1 mg/dl in HBV, HCV, and co-infected patients, respectively. While in the normal control group, the mean score of bilirubin was 0.76 ± 0.13 mg/dl.

3.1.6 Comparison of Bilirubin among Different Ethnic Groups

The mean scores of bilirubin in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 2.2 ± 1.32 , 2.2 ± 1.74 , and 2.1 ± 1.58 mg/dl, respectively, as illustrated in table 2. The mean scores of bilirubin in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were 2 ± 1.2 , 2 ± 1.3 , and 1.2 ± 1.8 mg/dl, respectively, as shown in table 3. The mean scores of bilirubin in Pushtoon, Saraiki, and Rajpoot co-infected patients were 3.2 ± 1.4 , 2.96 ± 2.28 , and 2.1 ± 1.2 mg/dl, respectively, as displayed in table 4. While in the normal control group, the mean score of bilirubin was $0.11\pm.13$ mg/dl, as shown in tables 2, 3, and 4.

3.1.7 Total Protein in Various Types of Hepatitis

The mean scores of total protein were 6.01 ± 1.5 , 5.8 ± 1.4 , and 5.5 ± 1.4 g/dl in HBV, HCV-infected, and co-infected patients, respectively. While in the normal control group, the mean score of total protein was 6.8 ± 2.0 g/dl (Table 5).

3.1.8 Comparison of Total Protein among Different Ethnic Groups

The mean scores of total protein in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 5.8 ± 1.7 , 6.1 ± 1.5 , and 6.5 ± 1.1 g/dl, respectively, as displayed in table 2. The mean scores of total protein in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were 5.9 ± 1.2 , 5.5 ± 1.5 , and

6.1±1.4 g/dl, respectively, as illustrated in table 3. The mean scores of total protein in Pushtoon, Saraiki, and Rajpoot co-infected patients were 4.9±1.6, 5.9±1.2, and 5.7±1.3 g/dl, respectively, as shown in table 4. While in the normal control group, the mean score of total protein was 6.8±2.0 g/dl as displayed in tables 2, 3, and 4.

3.1.9 Comparison of Blood Urea among Different Types of Hepatitis

The mean scores of blood urea were reported as 41.43±21, 39.21±20, and 48.25±21 mg/dl in HBV, HCV, and co-infected patients, respectively. While in the normal control group, the mean score of blood urea was 24.5±12 mg/dl (Table 5).

3.1.10 Comparison of Blood Urea among Different Ethnic Groups

The mean scores of blood urea in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 46±19.0, 46±26.4, and 42±8.98, respectively, as depicted in table 6. The mean scores of blood urea in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were 46±19, 43.8±16, and 43.35±11.5 mg/dl, respectively, as shown in table 7. The mean scores of blood urea in Pushtoon, Saraiki, and Rajpoot co-infected patients were 52±27, 48±21, and 43.6±13 mg/dl, respectively, as exhibited in table 8. While in the normal control group, the mean score of blood urea was 24.5±12 mg/dl as presented in tables 6-8.

Table 6. Renal function tests of Hepatitis B infected patients

Parameters	Ethnic groups with HBV						Normal Subjects		Normal range
	Pushtoon HBV		Saraiki HBV		Rajpoot HBV		Mean	SD	
	Mean	SD	Mean	SD	Mean	SD			
Blood Urea	46	22	46	28	42	7.4	24.5	6.7	10-50 mg/dl
Blood Creatinine	1.4	0.7	1.5	0.92	1.2	0.4	0.74	0.12	0.1-1.5 mg/dl

LFTs: liver function tests

Table 7. Renal function tests of Hepatitis C infected patients

Parameters	Ethnic groups with HCV infection								Normal range
	Pustoon HCV		Saraiki HCV		Rajpoot HCV		Control group		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Blood Urea	45.9	19.06	43.8	16.58	40.35	25.67	24.5	6.71	10-50 mg/dl
Blood Creatinine	1.5	0.87	1.26	0.64	1.37	0.96	0.74	0.12	0.1-1.5 mg/dl

LFTs: liver function tests

Table 8. Renal function tests of Co-infected patients

Parameter	Ethnic groups with Co-infection								Normal range
	Pushtoon ethnic		Saraiki ethnic		Rajpoot		Control group		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Blood Urea	52.2	27.6	47.9	21.86	43	12.9	24.5	6.71	10-50 mg/dl
Blood Creatinine	2.01	1.4	1.72	1.15	1.32	0.52	0.74	0.12	0.1-1.5 mg/dl

LFTs: liver function tests

3.2. Comparative Analyses of Biochemical Parameters

Comparison of Biochemical and hematological parameters among Hepatitis type (Type of Infection) wise, ethnicity-wise, age-wise, and gender-wise were described as follows.

3.3. Gender Wise

Gender-wise analysis of patients' biochemical parameters indicated that male patients infected with Hepatitis B, C, and co-infection have higher levels of parameters than female patients. The mean ALT was 378 IU/L in male patients infected with Hepatitis B, C, and co-infected, while females had a mean ALT of 313 IU/L (Figure 1).

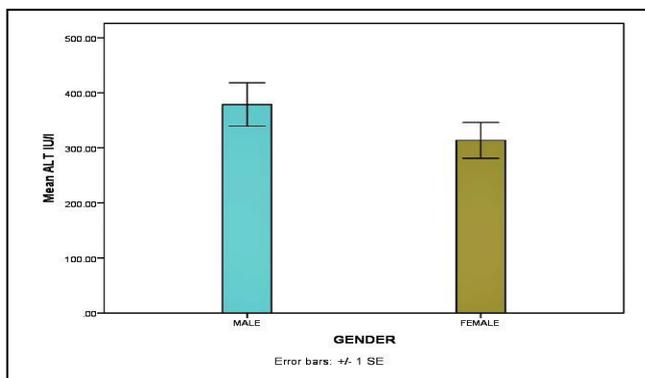


Figure 1. Mean Alanine transaminase vs. gender representative graph of Hepatitis B, C, and co-infected patients (n=200)

3.4. Liver Function Test (ALT) vs. Age-Wise Comparison

Age-wise patients were assigned to three groups. Patients in Groups 1, 2, and 3 were within the age ranges of 28-40, 41-50, and 51-60 years, respectively. A sharp rise in ALT was detected in patients in Groups 3, 2, and 1, respectively, as depicted in figure 2.

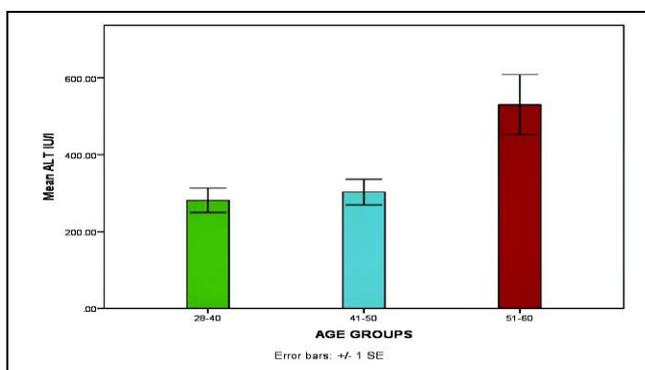


Figure 2. Liver function test Alanine transaminase vs. age wise comparison

4. Discussion

The present study aimed to evaluate and compare some biochemical parameters in HBV, HCV, and co-infected patients and establish the association between biochemical parameters and different types of Hepatitis infections in district Dera Ismail Khan. The study also compared various ethnic groups of Dera Ismail Khan District with regard to different types of hepatitis. When Hepatitis B and Hepatitis C infections were compared, it was observed that Hepatitis B has more severity level/ elevated parameters than Hepatitis C infection. Nonetheless, when co-infection was compared with Hepatitis B and Hepatitis C infections, it was observed that co-infection demonstrated more severity/elevated parameters, in comparison with single Hepatitis B or Hepatitis C infection.

High ALT values were noted in HBV, HCV, and co-infected patients, respectively. The mean scores of ALT were 376 ± 335 , 315 ± 265 , and 478 ± 519 IU/L in HBV, HCV, and HBV-HCV co-infected patients, respectively. Senturk, Tahan (18) also reported high ALT in Hepatitis B-infected patients, as compared to that in Hepatitis C-infected ones. In a similar vein Flisiak, Halota (19) indicated acute Hepatitis B infected patients with ALT > 10 times ULN, and 352 IU/L ALT levels in Hepatitis B infected patients higher than Hepatitis C. A recent study published in Korea presented that 70% of hepatocellular carcinoma (HCC) markers were diagnosed in Hepatitis B infected patients, while only 8% of HCC markers were diagnosed in Hepatitis C patients. The mean ALT was 431 ± 803 IU/L in co-infected patients which was much higher than single Hepatitis B and Hepatitis C infection.

Hepatitis progression is commonly more severe in HBV-HCV co-infected patients (20). Zaman, Asad (21) compared the mean ALT of 807.23 IU/L in Hepatitis B infection which was many times greater than Hepatitis C infection. The mean scores of ALT were 415 ± 309 , 390 ± 428 , and 324 ± 258 IU/L in Pushtoon, Saraiki, and Rajpoot ethnic groups with HBV, respectively. The

mean scores of ALT in Pushtoon, Saraiki, and Rajpoot HCV-infected patients were 324 ± 283 , 355 ± 273 , and 265 ± 243 IU/L, respectively. The mean scores of ALT were 518 ± 575 , 464 ± 579 , and 451 ± 410 IU/L in Pushtoon, Saraiki, and Rajpoot co-infected patients, respectively. Ethnicity wise study has suggested that Pushtoon ethnic group HBV-HCV infected with HBV and co-infection represented higher values of ALT and degree of severity, as compared to Saraiki and Rajpoot ethnic groups. While Saraiki ethnic group presented a dramatic rise in ALT, as compared to Pushtoon and Rajpoot ethnic groups.

Centers for Disease Control and Prevention (CDC) has presented a study on the association between various types of hepatitis and different ethnic group (i.e., non-Hispanic, Mexican American White, non-Hispanic Black, and others, which involved other Hispanics, Native Americans, and Asians). They also have pointed to changes in ethnicity of patients with Hepatitis (22). Higher values of total bilirubin were noted in HBV-HCV co-infection, as compared to single Hepatitis B and Hepatitis C infection, while among Hepatitis B and Hepatitis C, high values were reported in Hepatitis B than Hepatitis C. Singh, Farooq (23) had also presented the higher levels of bilirubin in HBV-HCV co-infection, Hepatitis B, and C, respectively.

The mean scores of bilirubin in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 2.2 ± 0.87 , 2.2 ± 1.5 , and 2 ± 1.4 mg/dl, respectively. The mean scores of bilirubin in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were 2 ± 1.2 , 2 ± 1.3 , and 1.9 ± 1.2 mg/dl, respectively. The mean scores of bilirubin in Pushtoon, Saraiki, and Rajpoot co-infected patients were 3 ± 1.4 , 2.9 ± 2.3 , and 2.1 ± 1.3 mg/dl, respectively. An ethnicity-wise study has suggested that patients infected with HBV and co-infection in Pushtoon ethnic group had a higher level of bilirubin, as compared to Saraiki and Rajpoot ethnic groups. Bilirubin values were slightly different and higher than the baseline or normal range.

The mean ALP of 358 mg/dl was noted in HBV which was greater than that in HCV. A similar study

conducted by Zaman, Asad (21) has also reported a mean ALP of 353.43 ± 1.30 . It seems that Hepatitis B has presented high values of ALP than Hepatitis C infection. The mean scores of ALP in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 308 ± 111 , 291 ± 84 , and 275 ± 92 mg/dl, respectively. The mean scores of ALP in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were 273 ± 128 , 305 ± 106 , and 301 ± 109 mg/dl, respectively. Finally, the mean scores of ALP in Pushtoon, Saraiki, and Rajpoot co-infected patients were 354 ± 104 , 374 ± 99 , and 345 ± 105 mg/dl, respectively. Pushtoon ethnic group presented higher ALP in HBV and HBV-HCV co-infection, while Saraiki ethnic group presented a huge rise in ALP, as compared to Pushtoon and Rajpoot ethnic groups.

The concentration of total protein which shows liver synthetic function was lower in HBV-HCV co-infection, HCV, and HBV, respectively. The mean scores of total protein in HBV, HCV, and co-infected patients were 6.1 ± 1.5 , 5.8 ± 1.4 , and 5.5 ± 1.4 g/dl, respectively. According to Singh, Farooq (23), all the biochemical parameters were higher among Hepatitis B, as compared to Hepatitis C. Li, Ma (24) has also observed hypo-proteinemia in Hepatitis B. The mean scores of total protein in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HBV were 5.2 ± 1.7 , 6.1 ± 1.5 , and 6.5 ± 1.1 g/dl, respectively. The mean scores of total protein in Pushtoon, Saraiki, and Rajpoot ethnic groups infected with HCV were 5.9 ± 1.2 , 5.5 ± 1.5 , and 6.1 ± 1.4 g/dl, respectively. Finally, the mean scores of total protein in Pushtoon, Saraiki, and Rajpoot ethnic groups co-infected patients were 4.9 ± 1.6 , 5.9 ± 1.2 , and 5.1 ± 1.3 g/dl, respectively.

The mean scores of blood urea in HBV, HCV, and co-infected patients were 45 ± 21 , 43 ± 20 , and 49 ± 21 mg/dl, respectively. The mean scores of blood creatinine in HBV, HCV, and co-infected patients were 1.4 ± 0.7 , 1.4 ± 0.8 , and 1.6 ± 1.1 mg/dl, respectively. Hepatitis B-related glomerular/renal impairment is due to the deposition of immune complexes in the glomerulus. Hepatitis B surface antigen (HBsAg), core

antigen (HBcAg), hepatitis B early antigen (HBeAg), and covalently closed circular DNA were involved in Hepatitis B-related glomerulonephritis (25). Okuse, Yotsuyanagi (26) has also reported that Hepatitis led to a reduction of proteinuria in hepatitis patients, especially children, with HBV-related membranous nephropathy.

The deposition formed in the sub-epithelial area can also occur in the sub-endothelial and mesangial region, directly proportional to the mass of the immune complexes and antigens. The low molecular weight of HBeAg 3×10^5 Da might be the reason for its capability to traverse the glomerular basement membrane, and therefore, the development of subepithelial immune deposits (27). Gender-wise analysis of patients' biochemical parameters pointed out that HBV, HCV, and co-infected male patients have a huge rise in parameters, as compared to female patients. Male and female patients infected with Hepatitis B, C, and Co-infection had mean ALT scores of 378 and ALT 313 U/l, respectively. Singh, Farooq (23) also reported some results similar to the study by Adoga, Gyar (28).

Regarding age, patients were assigned to three groups of 28-40, 41-50, and 51-60 years. Group 1 (28-40 years) infected with Hepatitis B, C, and dual Hepatitis B and C had a mean ALT of 281 ± 271 U/L. Group 2 (41-50 years) infected with Hepatitis B, C, and dual Hepatitis B and C had a mean ALT of 302 ± 288 U/L, while Group 3 (51-60 years) infected with Hepatitis B, C, and dual Hepatitis B and C had a mean ALT of 522 ± 566 U/L. The highest elevation of ALT was observed in Group 3, Group 2, and Group 1, respectively. Singh, Farooq (23) also reported similar changes in terms of age.

It is concluded that HBV-HCV co-infected patients were more seriously affected, as compared to single infection; therefore, mass vaccination and awareness programs should be initiated on an urgent basis. An ethnicity-wise study provided evidence that different ethnic groups showed different degrees of severity. There might be some genetic factors involved in the

fight against hepatitis B and C viral infection due to which all three ethnic groups demonstrated different degrees of severity. Further study at the genetic level may be needed to search for single nucleotide polymorphism at the gene level in the studied ethnic groups. The environment of the region or the social context may affect the degree of severity since in deprived areas, people are less aware of diseases. Further studies are needed to characterize HBV and HCV prevalent in Pakistan at the molecular level. Moreover, both host and viral factors associated with the molecular and cellular mechanisms of HBV and HCV infection in the Pakistani population need to be explored.

Authors' Contribution

Study concept and design: S. K. and M. A.

Acquisition of data: S. K. and Z. R.

Analysis and interpretation of data: M. A. and R. N.

Drafting of the manuscript: K. S. and A. K.

Critical revision of the manuscript for important intellectual content: B. O. and Z. S.

Administrative, technical, and material support: S. K., M.A, and R.N

Ethics

Ethical approval for all the experimental protocols of the study was taken from the Ethical and Research Board of Gomal University, Dera Ismail Khan, KPK, Pakistan.

Conflict of Interest

The authors declare that they have no conflict of interest.

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