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Identification of Predictive Factors in Chronic Hepatitis C Patients with Non-Infected Individuals: A Comparative Analysis

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MS and MI conceived the study, participated in the design and coordination of the study, writing of the manuscript. AR participated in the statistical analysis of the results. IA and SA helped in the writing of the manuscript. All authors read and approved the final manuscript.

Key words
Chronic hepatitis C virus, Host-virus factor, Predictive biomarkers, Patients clinical data

ABSTRACT

Hepatitis C virus is playing a key role in chronic liver disease all around the globe. Currently, in Pakistan, it is a huge burden on health and the economy, affecting about 6 to 8% of the overall population. HCV is dependent on the lipid metabolism of the patient to replicate and then disturb the blood cell count and liver enzymes to ultimately damage the liver. To distinguish the potential HCV patients from healthy individuals at an early stage is quite important for its control, which can be achieved by investigation of liver enzymes, complete blood count, and lipid profile of patients. In this study, 144 CHC and 20 controls were included. The serum of CHC patients was analyzed for HCV viral load and genotype. The liver function test, lipid profile, sugar levels, and complete blood count values were assessed in the laboratory. Receiver operating characteristics were used to find the biomarkers of infection. Logistic regression analysis revealed that liver enzymes (ALT, AST, ALP, γGT, albumin), blood cell count (globulin, platelets, MCHC RBC, WBC, monocytes% and lymphocytes%), lipid profile (HDL, LDL, VLDL, TC) were significant predictive factors in HCV infection. Similarly, ROC analysis suggested that ALT, AST, ALP, MCHC, TC, and VLDL variables have the potential to discriminate HCV infection from healthy individuals. Biochemical markers like liver enzymes, blood cell count, lipid profile are the main predictive factors of Chronic Hepatitis C patients as compare to the healthy group.

INTRODUCTION

The infectious hepatitis C virus (HCV) is a well-known global pathogen of chronic liver disease, with approximately 1.75 million new HCV infections and about 71 million people chronic disease (Dennis *et al*. 2021; Pawlotsky *et al*., 2018). Most of the infected patients (60-80%) develop chronic infections which lead to liver fibrosis, cirrhosis, and ultimately to hepatocellular carcinoma (HCC) in the future (Bray *et al*., 2018; Meringer *et al*., 2019). Among the native population of Pakistan, the prevalence rate of chronic hepatitis C (CHC) is between 6 to 8 percent, representing more than 10 million people (Hamid *et al*., 2004; Samo *et al*., 2020). However, the burden of CHC infection fluctuates in different municipalities of the country. The highest reported rate is in Gujranwala (23.8%), then comes in Karachi (20%) and Lahore (16%) (Akhtar and Moatter, 2007; Muhammad and Jan, 2005; Siddique *et al*., 2020). In Pakistan, the majority of patients infected with HCV have genotype 3a, then 3b, and 1a respectively (Idrees and Riazuddin, 2008; Khan *et al*., 2020).

HCV has positive-strand RNA and belongs to the family Flaviviridae. The virus replicates in the liver of an infected person (Bartenschlager *et al*., 2011; Choo *et al*., 1989). HCV has been categorized into seven recognized genotypes according to the sequence of whole genomes (Simmonds *et al*., 1994; Smith *et al*., 2014). HCV infection spreads in humans as a result of exposure to contaminated blood, injectable drug use (IDU), blood transfusions, unsafe medical procedures, unsterilized needles, and vertical transmission (Benova *et al*., 2014; Maheshwari and Thuluvath, 2010).

To establish infection in the host, HCV relies on lipid metabolism to replicate. The virus combines with lipoproteins to form a structure called lipovirus particles (LPV), which is released by hepatocytes (Benova *et al*.

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2014; Fukuhara *et al*. 2015). During cell adsorption, entry, and virion maturation, HCV viruses use very low density lipoprotein (VLDL) and low-density lipoprotein (LDL) receptors (Crouchet *et al*., 2017).

Simple, easy-to-use, non-invasive methods that can identify HCV infection at an early stage will greatly help to control the disease. Several studies have been attempted to identify blood-based biomarkers to forecast hepatic dysfunction in HCV-infected individuals. During infection changes in aminotransferase enzyme (Quaranta *et al*., 2021) lipid metabolism (de Souza Lacerda *et al*., 2018; Eletreby *et al*., 2021) and blood count abnormalities have been observed in HCV-infected patients (Cacoub *et al*., 2000; Nikiforuk *et al*., 2021). In most HCV-infected individuals, the exact mechanisms of abnormal blood lipid levels and peripheral blood cell counts were unidentified. The study's key objective was to investigate blood-based liver enzyme, complete blood count, and lipids profile that can distinguish potential HCV infected patients from the non-infected population.

MATERIALS AND METHODS

Ethics statement

Approval has been taken from the Ethical Committee of CEMB, University of the Punjab for the current study. After proper guidance, signed consent forms were obtained before collecting the samples from patients.

Patients

Patients infected with CHC were selected for the study. Patients who had previously received treatment, co-infection, and genetic disease or alcohol history were excluded from the study. Patients demographics data (gender, age, BMI) was collected and serum was analyzed for virological (viral load, genotype) and clinical (liver function test, lipid profile, sugar level) analysis.

Laboratory assessment

Blood samples obtained from enrolled patients were used to investigate the liver function enzymes including aminotransferase such as [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], alkaline phosphatase (ALP), globulin (Glb), gamma-glutamyl transferase (γ-GT), serum total protein (TP), albumin (ALB) and total bilirubin (TB). Triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very low-density lipoprotein cholesterol (VLDL-c), and total cholesterol (TC) were all examined for the lipid profile. Complete blood cell counts such as lymphocyte, monocyte, white blood cell (WBC), and red blood cell (RBC) of patients were also measured according to the manufacturer's instructions. Sysmex XE-2100 automated haematology analyzer (Sysmex Corp, Kobe, Japan) was used to determine the complete blood cell counts.

HCV viral load and genotyping detection

The patient's HCV viral concentration was measured by performing a real-time PCR (Cepheid, Smart Cycler II) as described in the manufacturer's manual by using serum samples. In this system, HCV viral load of lower than 250 IU/ ml has been considered as negative. The HCV genotype was performed as described by Idrees (2008).

Statistical analysis

Clinical data of HCV infected and healthy controls are compared by using the *stude*nt's T-test. The Chi-square test is used to analyze the categorical variables. Results are described as mean ± standard deviation. The relationship between clinical and virological variables is determined by applying correlation analysis. Logistic regression is used to predict the variables which are associated with HCV infection. ROC analysis is performed for the identification of potential biomarkers of infection. The statistical software SPSS (22.0) is used to perform statistical analysis of data. A P-value lower than 0.05 is considered significant.

RESULTS

Patients baseline characteristics

Baseline values of enrolled patients have been summarized in Table1 like demography, clinical, and molecular virological characteristics. A total number of 144 treatment-naïve CHC patients were participated in this study, out of which an equal ratio of males and females was considered for study i.e 72 each. It is noted that the mean age of CHC patients was 35.92 ± 9.66 ranging from 18 to 60 years. The patients considered for the study had HCV 3a genotype.

A remarkable difference in values of HCV-infected and non-infected individuals were observed especially in viral loads, liver enzymes, lipid profile, and complete blood count.

Baseline variables in HCV infected compared control samples

To see if there was a difference between HCV infected and control subjects, an independent samples T-test was used. The results revealed that liver function enzymes levels such as aminotransferase (ALT, AST), ALP, γ-GT, albumin, and globulin levels differed significantly ($p < 0.05$) between infected and control subjects. Platelet counts and monocytes% were significantly lower in HCV patients but the level of lymphocytes was significantly higher as compared to the control subjects. Another significant

difference was observed in VLDL which is higher in HCV patients but the HDL was lower in the same as compared to controls. Similarly, globulin was significantly lower in HCV patients in comparison with the control group (Table I).

Table I. Baseline characteristics of patients with chronic hepatitis C.

Parameters	Healthy controls	Chronic hepatitis C patients
Gender (M/F)	10/10	72 / 72
Age (years)	41 ± 2.8 (33-45)	36 ± 9.7 (18-60)
BMI	22.0 ± 1.4 (19.5-23.8)	24.0 ± 2.7 (18.4-29.5)
Viral load (IU/ml)	-	9.0*10^5 ± 1.5*10^6 (4.078*10^3-7.389*10^5)
HCV Genotypes	-	3a
ALT(IU/L)	29 ± 3.9 (20-34)	59 ± 29.2 *** (13-141)
AST(IU/L)	28 ± 4.6 (20-35)	48 ± 24.1 *** (10-134)
AST/ALT ratio	0.98 ± 0.21 (0.59-1.38)	0.85 ± 0.36 (0.38 – 2.00)
ALP(IU/L)	106 ± 19.7 (87-143)	203 ± 60.5 *** (88-460)
Alb (g/dl)	4.1 ± 0.2 (3.8-4.3)	4.5 ± 0.6 ** (3.2-5.8)
Glb (g/dl)	2.9 ± 0.3 (2.5-3.3)	2.6 ± 0.4 *** (1.5- 3.6)
Tp (g/dl)	7.2 ± 0.5 (6.2-8.0)	7.2 ± 0.5 (6.2 – 8.1)
BT (g/dl)	0.8 ±0.1 (0.7-0.9)	0.75 ± 0.2 (0.4 – 1.6)
γ-GT (IU/L)	23 ± 7.5 (12 -36)	29 ± 15.0 * (8 - 91)
TC (mg/dl)	150.2±14.1 (90.5-180.2)	175.33 ± 23.53 (112 - 205)
HDL-c (mg/dl)	49 ± 2.3 (46 -52)	38 ± 6.4 *** (27-56)
LDL-c (mg/dl)	97 ±16.0 (75 -122)	112 ± 30.2* (60 - 204)
VLDL-c (mg/dl)	16 ± 3.0 (12 - 21)	28 ± 6.0 *** (16 - 50)
Platelets (10^9/L)	267 ± 36 (210-340)	226 ± 75* (110 - 459)
Lymphocytes(%)	33 ± 4 (29-40)	40 ± 10** (25 - 75)
Neutrophils(%)	59 ± 8.6 (45-69)	56 ± 9.8 (30 - 72)
Monocytes (%)	4 ± 1.6 (2-7)	2 ± 0.8*** (1 - 4)
Eosinophils(%)	4 ± 1.3 (2-6)	2 ± 1.2 (1 - 7)
Hb	13.3 ± 1.0 (11.6-15.2)	13.3 ± 1.8 (9.2 - 17.8)
MCV	90 ±1.4 (88 - 92)	84 ± 5.2*** (72 - 92)
MCH	29 ± 1.3 (27 - 31)	29 ± 3.5 (19 - 36)
MCHC	31± 0.8 (30-32)	34 ± 2.9*** (23 – 41)
RBC	5 ± 0.4 (4.3-5.5)	4.6 ± 0.7* (3.2-7.2)
WBC	8.4 ± 1.4 (6.1-10.4)	6.7 ± 2.0*** (4.4-16.4)

Mean±SEM. Students test; * P<0.05, ** P<0.01, ***P<0.001. Data were expressed as mean ± SD. BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; albumin; Glb, globulin; γ-GT, gamma-glutamyl transferase; TC, total cholesterol; TG, triglycerides; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL-c, very low-density lipoprotein cholesterol; Hb, hemoglobin concentration; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; PCV, packed cell volume; MCHC, mean corpuscular hemoglobin concentration.

Table II. Logistic Regression Analysis of predictors in CHC patients compared with healthy controls.

Variables	B	S.E.	P	Exp(B)	95% C.I. for EXP(B)	
					Lower	Upper
ALT	.062	.017	.000	1.064	1.030	1.100
AST	.067	.020	.001	1.070	1.029	1.112
ALP	.066	.013	.000	1.068	1.042	1.095
γGT	.080	.035	.024	1.083	1.011	1.161
Globulin	-3.762	.924	.000	.023	.004	.142
Serum albumin	1.454	.519	.005	4.280	1.547	11.842
Platelets	-.007	.003	.021	.993	.987	.999
HDL	-.376	.078	.000	.686	.589	.800
LDL	.022	.010	.036	1.022	1.001	1.043
VLDL	.640	.139	.000	1.896	1.443	2.492
MCHC	.473	.126	.000	1.605	1.253	2.057
RBC	-.765	.327	.019	.465	.245	.882
WBC	-.276	.104	.008	.759	.620	.930
Lymphocyte%	.126	.043	.003	1.134	1.043	1.233
Monocyte%	-1.396	.304	.000	.248	.137	.449
TC	.047	.013	.000	1.048	1.022	1.074

For abbreviations, see Table I.

Logistic regression model

In order to recognize the aspects associated with HCV infection, a regression analysis was performed known as binary logistic regression analysis. The HCV infection versus healthy individuals was used as a dependent variable and clinical data of patients was used as an independent factor. The analysis revealed that ALT, AST, ALP, γGT, serum albumin, globulin, platelets, HDL, LDL, VLDL, MCHC, RBC, WBC, TC, monocyte%, and lymphocyte% were significant predictors of HCV infection Table II.

ROC analysis

ROC analysis with an area under the curve was considered to determine the biomarker value of study variables. The analysis revealed that ALT, AST, ALP, MCHC, TC, and VLDL variables have the potential to discriminate HCV infection from healthy individuals in Table III and Figure 1.

Table III. Receiver operator characteristic (ROC) Analysis in CHC patients compared with healthy.

Test result variable(s)	Area	P	95% confidence interval	
			Lower bound	Upper bound
ALT	0.803	.000	.715	.891
AST	0.793	.001	.705	.881
ALP	0.952	.000	.912	0.992
VLDL	0.982	.000	0.961	1.000
MCHC	0.911	.000	.851	.971
TC	0.849	.000	.773	.924

For abbreviations, see Table I.

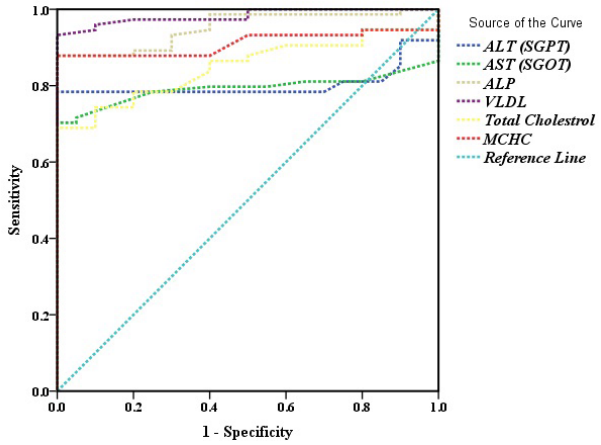

Fig. 1. Receiver operator characteristic (ROC) Analysis in CHC patients compared with healthy controls to assess the diagnostic potential of biochemical markers.

DISCUSSION

A great breakthrough has been made in CHC therapy in recent years. Identification of CHC infection in patients is very important because most infected individuals are unaware of their infection (Ghany *et al*., 2020). Timely identification of HCV infection is crucial to obtain the benefits of therapy. In the present investigation, we compared the liver enzyme, lipid profile, and blood count in CHC patients and healthy individuals to find the effect of HCV infection on biochemical markers. The relationship and predictive performance of biomarkers was also investigated. Liver enzymes (ALT, AST, ALP, and γ-GT) are an indicator of liver injury and their levels changes in the presence of viral disease. These enzymes are abundant in the liver and leak from the liver into the blood during hepatic injury. These enzymes levels can be used to screen for possible liver damage during HCV infection (Akkaya *et al*., 2007; Sugimoto *et al*., 2018). According to epidemiological research data, there is a link between liver enzymes ALT, AST, γ-GT levels and liver-related disease and mortality (Kunutsor *et al*., 2014). It has been reported that liver enzyme (ALT) levels increased in HCV patients (Giuffrè *et al*., 2020; Hajarizadeh *et al*., 2016). Logistic regression analysis demonstrated that the ALT, AST, ALP and γ-GT were associated with disease progression in HCV infection. Thus, in HCV infection these enzymes have predictive value. In our study, we have been found an encouraging correlation between ALT levels and the duration of HCV infection. Previous literature showed that many studies have failed to find such correlations (Haydon *et al*., 1998).

Blood counts can be used to check for suspected liver damage during HCV infection, according to studies. Previous data revealed that the Hepatitis C virus replicates in peripheral blood cells, and patients with clinical hepatitis C virus infection have abnormal blood cell counts (Lerat *et al*., 1996; Nikiforuk *et al*., 2021; Streiff *et al*., 2002). These blood cell helps in an immune response, such as fighting to an infection. The low blood cells mean that body has a weak immune response against the infection. In our study, we observed the effect of HCV infection on peripheral blood count in infected patients as compared to the control group. Our data showed that the mean platelet count, monocyte%, RBC's and WBC's in the CHC infected group were significantly less as compared to the control group. Our investigation indicates a significant association of HCV infection with low platelets count, monocyte %, RBC's and WBC's in the Pakistani population. These findings help the practitioners to consider the blood count for screening in CHC patients. Data from the literature showed that low platelet count, monocyte %, RBC and

WBC have been associated with a number of markers of liver disease like serum bilirubin level, globulin and serum albumin (Bashour *et al*., 2000; Streiff *et al*., 2002).

Lipid metabolism has an important impact in HCV infection and is well described. It promotes the entry of HCV into the liver by binding to VLDL or LDL receptors (Cheng and Li, 2003; Wünschmann *et al*., 2000). In our study, chronic HCV patients have significantly lower values of serum lipids as compare to controls subjects. Several studies have found that serum lipid levels in HCV-infected patients are dysregulated, particularly low levels of LDL (Kuo *et al*., 2011). LDL and VLDL levels were high in HCV patients as compared to controls. HCV infection played role in lower cholesterol levels. Our data is not dependable on previous studies that suggested that hepatitis C exceptionally has an association with decreased LDL and cholesterol (Corey *et al*., 2009; Dai *et al*., 2008; Marzouk *et al*., 2007). High levels of LDL and VLDL are linked to an increased risk of heart disease. A few studies have found that people with persistent HCV infection had a higher risk of developing ischemic heart disease (Kakinami *et al*., 2013; Shahid and Rehman, 2020). Our study showed that serum lipid profile is predictors of CHC infection. There was a significant difference in levels of HDL, LDL, and VLDL in CHC patients. HDL level was significantly lesser in CHC patients than in the comparison group. On the other hand, CHC patients have significantly higher values of LDL and VLDL. These findings indicate that plasma lipids are involved in the development of HCV infection.

Finally, in our study, a ROC analysis has been performed, before considering these biochemical factors as noninvasive biomarkers of HCV infection as compared to healthy individuals. This analysis has been used to assess the diagnostic value of any biomarker and helps clinicians for a suitable treatment for diseased patients (Gu *et al*., 2008; Obuchowski and Bullen, 2018; Zheng *et al*., 2002). In our recent study, the ROC curve analysis disclosed that biochemical markers (ALT, AST, ALP, VLDL, MCHC, TC), had an AUC of 0.803, 0.793, 0.952, 0.982, 0.911, 0.849 which proved that these subsets of biochemical marker have a discrimination power between CHC patients and control group.

In conclusion, our study showed that liver enzymes (ALT, AST, ALP, γGT, albumin), lipid profile (HDL, LDL, VLDL, TC) and blood cell count (platelets, MCHC, RBC, WBC, monocytes% and lymphocytes%), are predictive biomarkers in HCV infection and an association has been observed between these variables and duration of HCV infection. ROC analysis showed that biochemical markers like ALT, AST, ALP, MCHC, TC and VLDL are diagnostic biomarkers between hepatitis patients as compare to the normal control group. Our data is obtained from the limited number of patients and controls and to find further positive correlations between these predictive biochemical markers and HCV, a vast study is recommended.

Statement of conflict of interest

The authors have declared no conflict of interest.

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