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# ASSOCIATION OF HCV VIRAL LOAD WITH HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN HEPATITIS C PATIENTS, PAKISTAN

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**Abstract**

The Hepatitis C viral infection contributes to a significant health burden worldwide. In Pakistan, about 6% of individuals are affected by HCV. Acute hepatitis is usually asymptomatic and rarely causes symptoms such as jaundice, fatigue, fever, and nausea. Around 85% of people affected with acute HCV initially may develop chronic disease. This study aims to find the effect of HCV viral load in the de-arrangements of triglycerides levels, ALT levels, total cholesterol levels, uric acid levels, hemoglobin, and WBCs quantity. After the HCV diagnosis with q-PCR, patients' blood samples were collected in EDTA and Gel tubes to estimate hematological, biochemical, and lipid profile parameters. The estimation of hemoglobin (Hb) and WBCs (white blood cells) was done by the hematological analyzer and the estimation of triglycerides, cholesterol, uric acid, and ALT levels was done by micro lab 300. Simple linear regression and persons correlation show a significant positive correlation of viral load with triglycerides ($p=0.008$), ALT ($p=<0.001$), and WBCs ($p<0.001$). Uric acid ($p=0.304$) and total cholesterol ($p=0.76$) shows a non-significant positive correlation. However, hemoglobin ($p=0.857$) shows a non-significant negative correlation with HCV viral load.

**Keywords:** Hepatitis C, Lipid Profile, ALT, WBC.

## INTRODUCTION

The word hepatitis means infection of the liver. The Hepatitis C virus contributes to a major health burden worldwide, about 130–170 million people are suffering from it (Dubuisson and Cosset, 2014). The effect of HCV viral load on hematological and biochemical parameters in hepatitis C patients is an interesting area to be explored. HCV is an enveloped and positive-sense RNA virus of approximately 9400 nucleotides. It is a

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member of the Flaviviridae family and contains structural, non-structural, and regulatory proteins (Roger et al., 2021). HCV's six (1-6) key genotypes have been acknowledged, and somewhat characterized. The subtypes are represented by letter designations (a, b, c, and so on). Thus, types 1a, 1b, 2a, and 2b are the most known or common HCV types. More than 50 subtypes have been described, and their nomenclature is unclear (Paraskevis et al., 2021). The Hepatitis C infection can be acute or chronic. Infection caused by HCV is acute hepatitis in about 20% of instances. Patients with acute hepatitis are typically asymptomatic or suffer from a non-specific clinical illness marked by weight loss, fatigue, malaise, and anorexia. Jaundice is prevalent in about 30% of symptomatic adults suffering from HCV (Modi and Liang, 2008). ALT levels may rise 10-fold in patients with HCV within days of acquisition of HCV. Around 70-85% of patients infected with acute HCV can develop chronic HCV later (Ghany and Morgan, 2020). Due to their asymptomatic or mild, nonspecific symptoms, the majority of people are unaware that they have this illness. Fatigue is most frequently observed in HCV patients (Blackard et al., 2008). Chronic patients with HCV further lead to advanced liver cirrhosis, steatosis, hepatocellular carcinoma, and fibrosis (Thomas, 2013). Only 20–30% of HCV-infected patients go on to acquire liver cirrhosis, and only 1-4 percent of cirrhotic patients get HCC each year (Gower et al., 2014). Over 58 million individuals are infected with chronic infection of HCV and each year 1.5 million new cases are reported. These numbers continue to vary widely across the globe (WHO, 2022). With an estimated 2.2% global prevalence of HCV infection, there are 130,000,000 HCV-positive people worldwide (Perz et al., 2006). Although the prevalence of HCV varies among Asia's nations, its overall prevalence is thought to be just over 2%. In Pakistan, about 6% of individuals are infected with HCV. According to studies about 10 million individuals in Pakistan are infected with hepatitis which makes it the second-highest HCV burden after Egypt (Umer and Iqbal, 2016) IDU (Injection drug use) is a well-established risk factor for HCV transmission. Sharing contaminated needles, syringes and other injection equipment can transmit the virus (Grebely et al., 2016). Hepatitis C can be transmitted through sexual contact but is much less capable than other sexually transmitted viruses, including human immunodeficiency virus (HIV) and hepatitis B virus. Blood transfusions are also a well-known way of transmission for hepatitis C virus infection (Sy and Jamal, 2006). De-arrangements in biochemical parameters and hematological parameters occur through HCV viral load concentration in hepatitis C patients. Higher HCV RNA levels are associated with increased serum triglyceride, total cholesterol, and low-density lipoprotein levels (Hsu et al., 2009). In advanced liver fibrosis, uric acid levels decrease in patients with chronic hepatitis C. However, uric acid levels are different when there are other manifestations seen like obesity, co-morbidity, etc. (Jang et al., 2018). There are no alterations seen in bilirubin and creatinine levels in HCV patients but there were increased levels of AST and ALT (Ibrahim et al., 2016). HCV infection also affects the hematological parameters. The white blood cell counts (WBCs) have been raised in HCV-affected patients (Tsai et al., 2015). The hemoglobin quantity has increased due to medication and showed a significant correlation with HCV viral load (Chen et al., 2020). Hence

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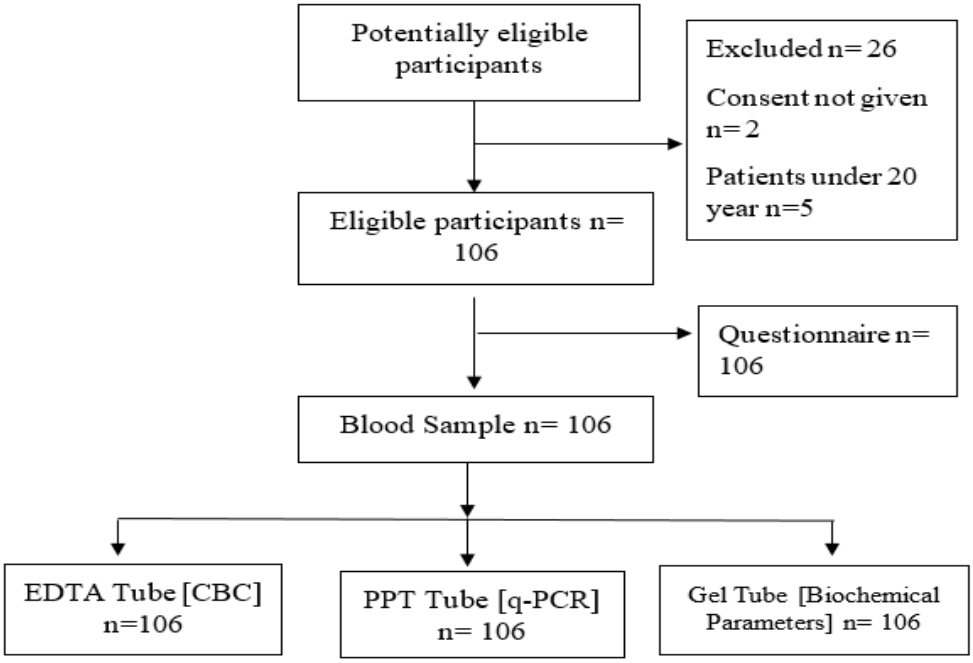
present study was undertaken to see the effect of HCV viral load on hematological and biochemical parameters.

## MATERIALS & METHODS

All the patients with written informed consent were enrolled in the study from January 2023 to June 2023 at Benazir Bhutto Hospital Rawalpindi. This research was approved by the ethical committee of Abasyn University. Data was collected with the informed consent of the patients. A total of 106 Hepatitis C patients were included in this study. Patients diagnosed with HCV, with an age limit >20 and <80, and with a Hepatitis history of at least 6 months were included in this study. A structural interviewing questionnaire was designed to collect the patients' clinical history and demographic information by informed consent. After filling out the questionnaire patient's blood sample was collected in PPT (for qPCR), EDTA (for CBC), and Gel tube (for uric acid, ALT, total cholesterol, and triglycerides). Estimation of HCV viral load was done through qPCR.

ALT levels, uric acid levels, total cholesterol levels, triglycerides levels, Hb quantity, and WBCs quantity were also measured. The obtained data was analyzed with SPSS and the normality of data was also checked through the Shapiro-Wilk test, skewness, and kurtosis. Statistical analysis included descriptive analysis, frequency, Pearson's Correlation, and Simple Linear Regression test to see the relationship of HCV viral load with hematological (WBCs & Hb) and biochemical parameters (ALT, Total Cholesterol, Triglycerides, and uric Acid). A p-value <0.05 was considered statically significant.




The study was done according to the Declaration of Helsinki principles, informed consent, dignity, autonomy, integrity, privacy, and confidentiality were carefully considered. The

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tests were performed under the PNCA registered lab and with their guidelines and regulations.

**Miro Lab 300 Procedure:**

- Prepare the working reagent for desired parameter.
- Pipette the working reagent (quantity as required) and Sample (as required).
- Mix and incubate.
- Run standard blank first and now machine is ready for test measurement.
- Aspirate the mixing by the analyzer and start measurement.
- Note the results.

**Q-PCR Procedure:**

- Extract and purify DNA or RNA from sample (Blood).
- Reverse transcribe RNA into complementary DNA (cDNA) using reverse transcriptase. (In RNA case).
- Prepare a reaction mixture with DNA template, primers, a DNA polymerase with exonuclease activity, and fluorescent dyes or probes.
- Distribute the reaction mix into PCR tubes or plates. And include appropriate controls.
- Measure fluorescence at each cycle to monitor DNA amplification.

## RESULTS

Of a total of 106 patients, 52.8% were females and 47.2% were males. 93.4% were married, 90.6% were non-smokers and 67% had no family history of hepatitis C (Table 1). In the present study, 51% (n = 54) of patients were in the age bracket 41-60 and 39% (n = 41) of patients were 21–40 years of age (Fig 1). Fatigue was the most common clinical finding among studied patients followed by fever (Fig 2). Samples for this study were collected from different areas; among them, Rawalpindi has the highest percentage 84.9% (Fig 3). Most of the patients included in the study had 1-6 months of hepatitis history, about 64.2% (Fig 4).

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**Table 1.** ***Demographic Characteristics of Studied Patients***

| Characteristic | Frequency | Percentage |
|---|---|---|
| **1-Gender (n =106)** | | |
| Male | 50 | 47.2% |
| Female | 56 | 52.8% |
| **2-Marital Status (n =106)** | | |
| Married | 99 | 93.4% |
| Un-married | 07 | 6.6% |
| **3-Smoking Status (n =106)** | | |
| Yes | 10 | 9.4% |
| No | 96 | 90.6% |
| **4-Family History (n =106)** | | |
| Positive | 35 | 33% |
| Negative | 71 | 67% |

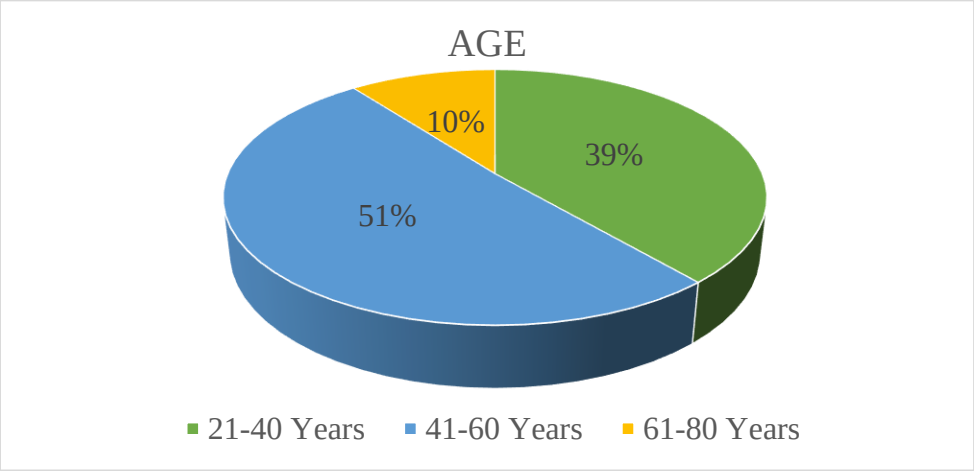


**Figure 1:** ***Age of the Studied Patients***

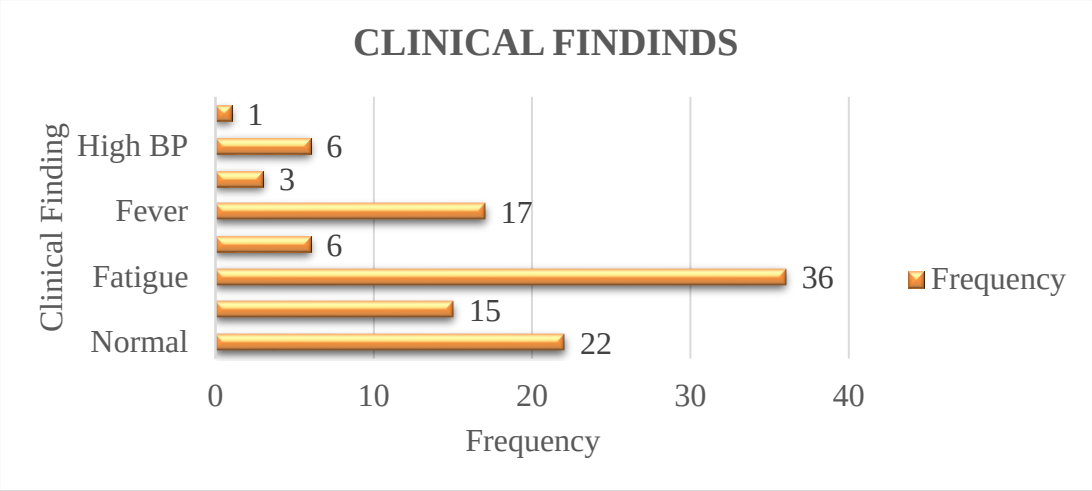


**Figure 2:** ***Clinical Findings of Studied Patients***

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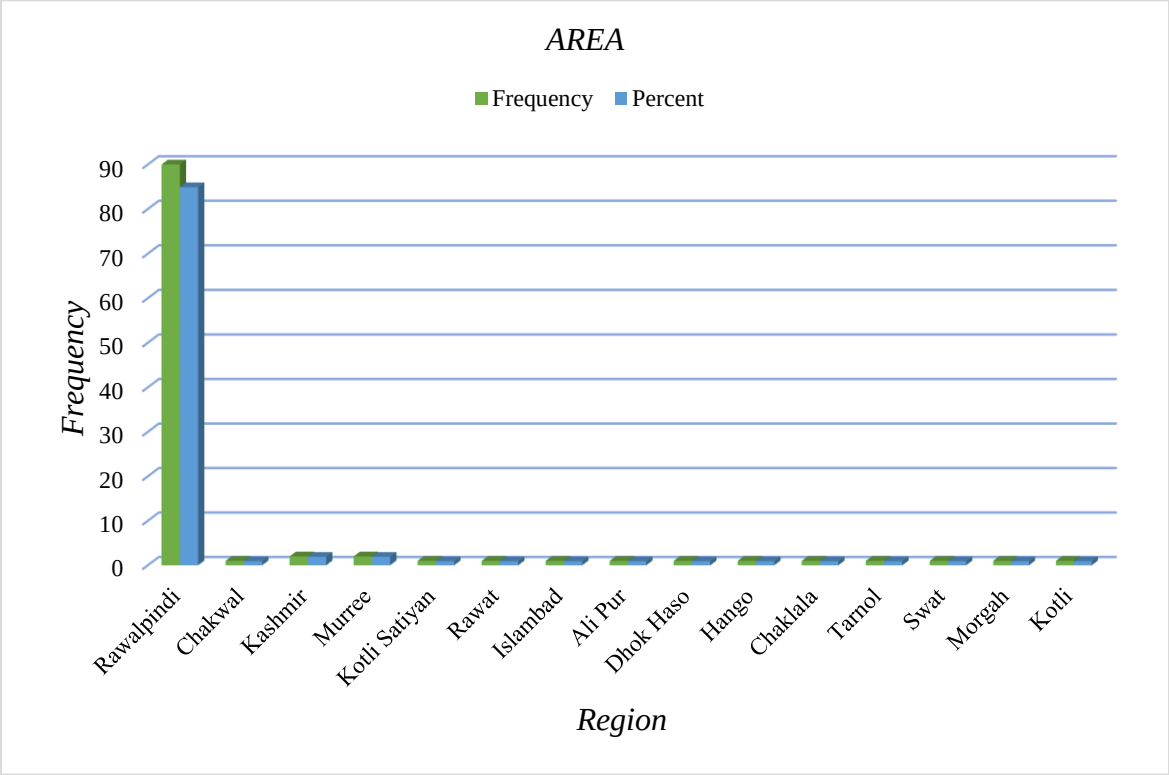


**Figure 3: *Area of Studied Patients***

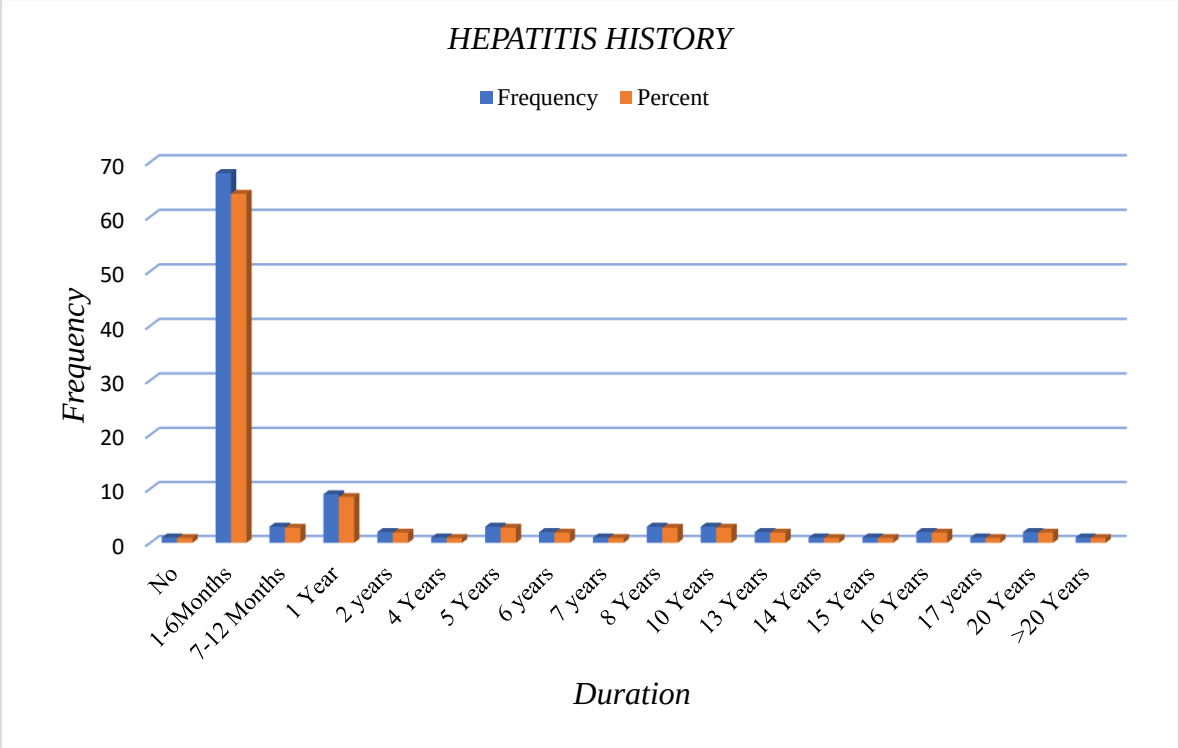


**Figure 4: *Hepatitis History of Studied Patients***

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**Table 2: Descriptive analysis of continuous data shows the mean value and standard deviation of the variables of interest. All continuous variables were normally distributed.**

| Variable Name | M | SD | Skewness | Kurtosis |
|---|---|---|---|---|
| Viral Load | 3,553,804.10 | 2688479.83 | 0.78 | -0.74 |
| ALT | 93.42 | 57.42 | 1.20 | 1.37 |
| Total Cholesterol | 214.37 | 40.27 | 0.35 | -0.81 |
| Triglycerides | 181.33 | 63.94 | 0.81 | -0.41 |
| WBCs Finding | 18736.16 | 8201.16 | 1.10 | 0.24 |
| Uric Acid | 4.75 | 1.34 | 0.06 | -0.64 |
| Hemoglobin | 11.90 | 2.25 | -0.11 | -0.36 |
| *Note:* M= Mean, SD= Standard Deviation, ALT=Alanine Transaminase, WBCs= White Blood Cells, and Normal Skewness and Kurtosis are within +2 & -2. | | | | |

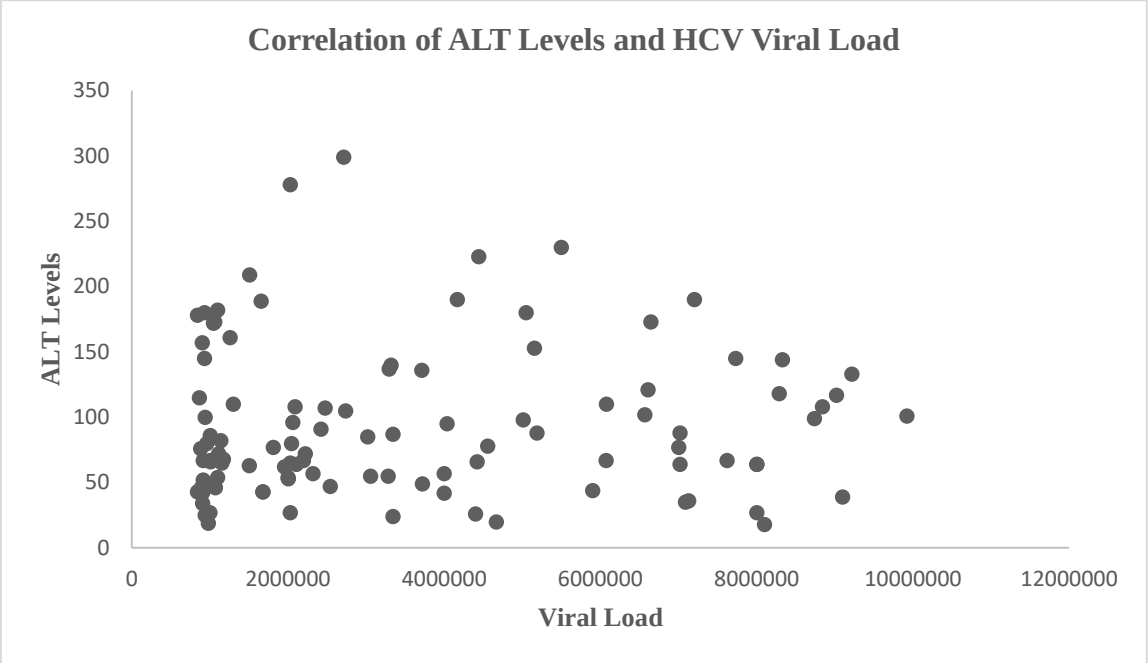


**Figure 5: *Correlation of ALT Levels and HCV Viral Load*. The Pearsons correlation analysis revealed a significant strong positive correlation between viral load and ALT levels (r = .346**, p < .01).**

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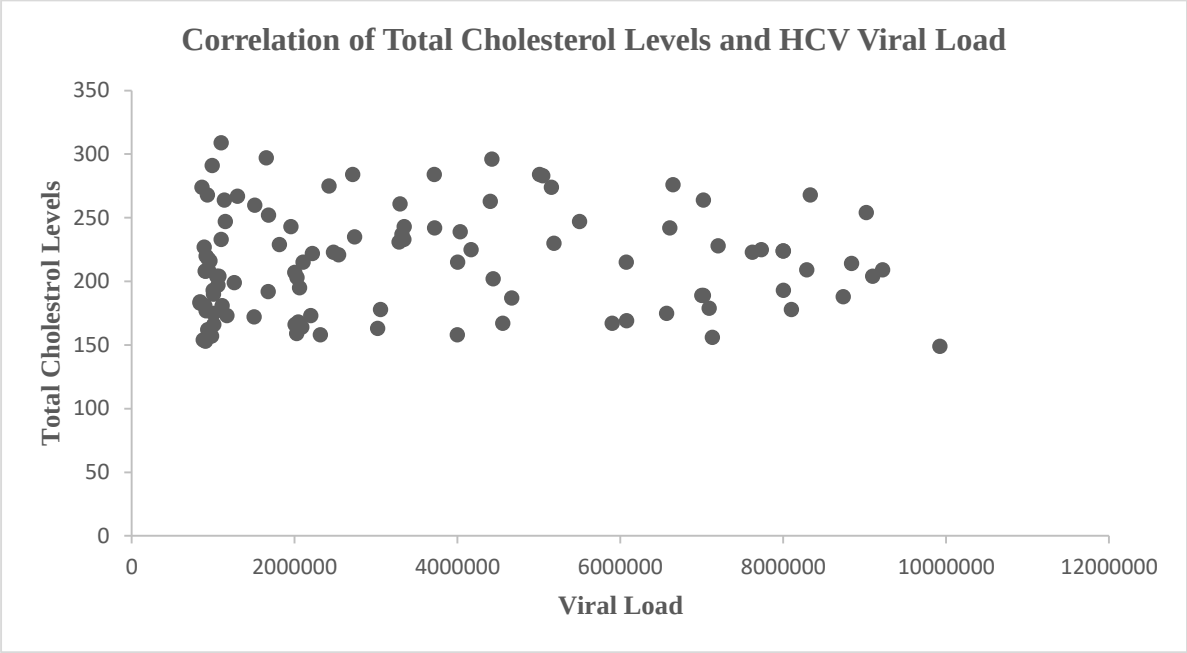


**Figure 6: *Correlation of Total Cholesterol Levels and HCV Viral Load.* The Pearsons correlation analysis revealed a non-significant positive correlation between viral load and total cholesterol levels (r = .137, p 0.076).**

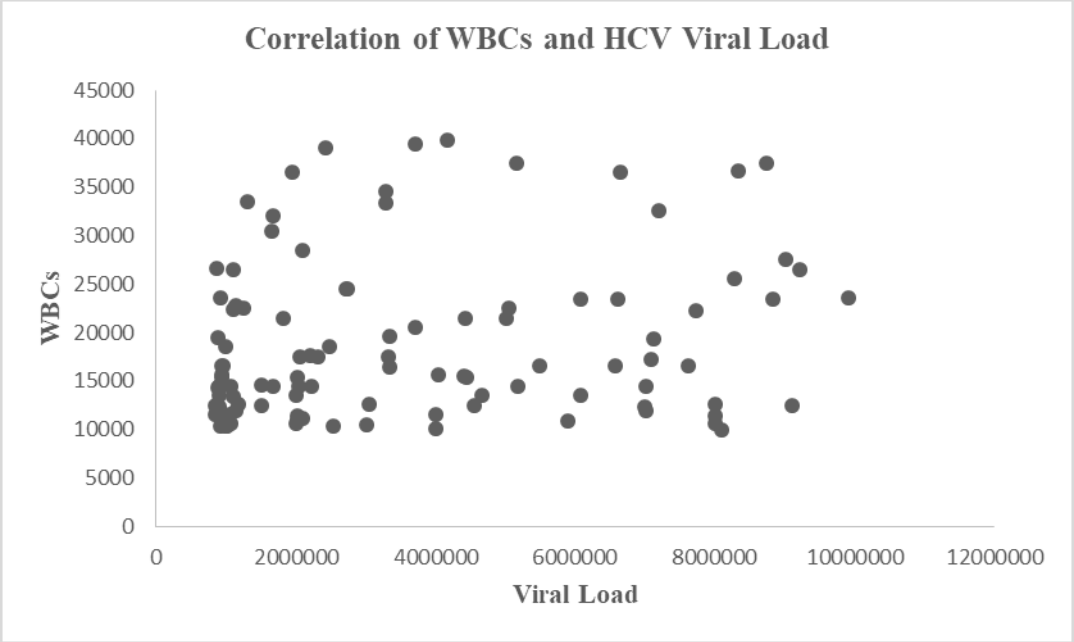


**Figure 7: *Correlation of WBCs and HCV Viral Load.* The correlation analysis revealed a significant strong positive correlation between viral load and WBCs quantity (r = .832**, p < 0.01).**

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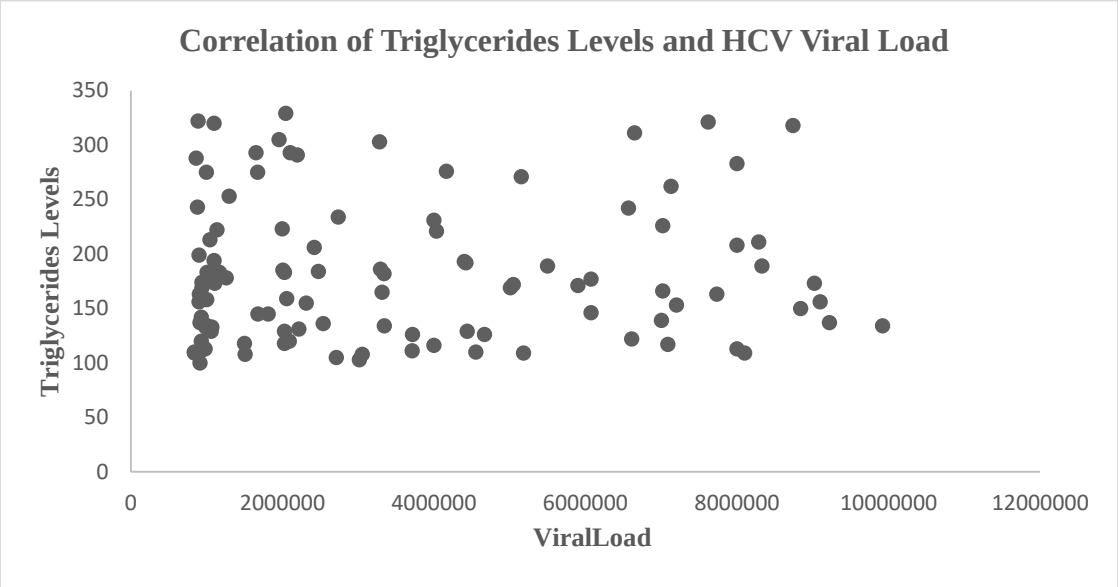


**Figure 8:** ***Correlation of Triglycerides Levels and HCV Viral Load.*** **This study also found a significant positive correlation between viral load and triglycerides (r = .257**, p = .008).**

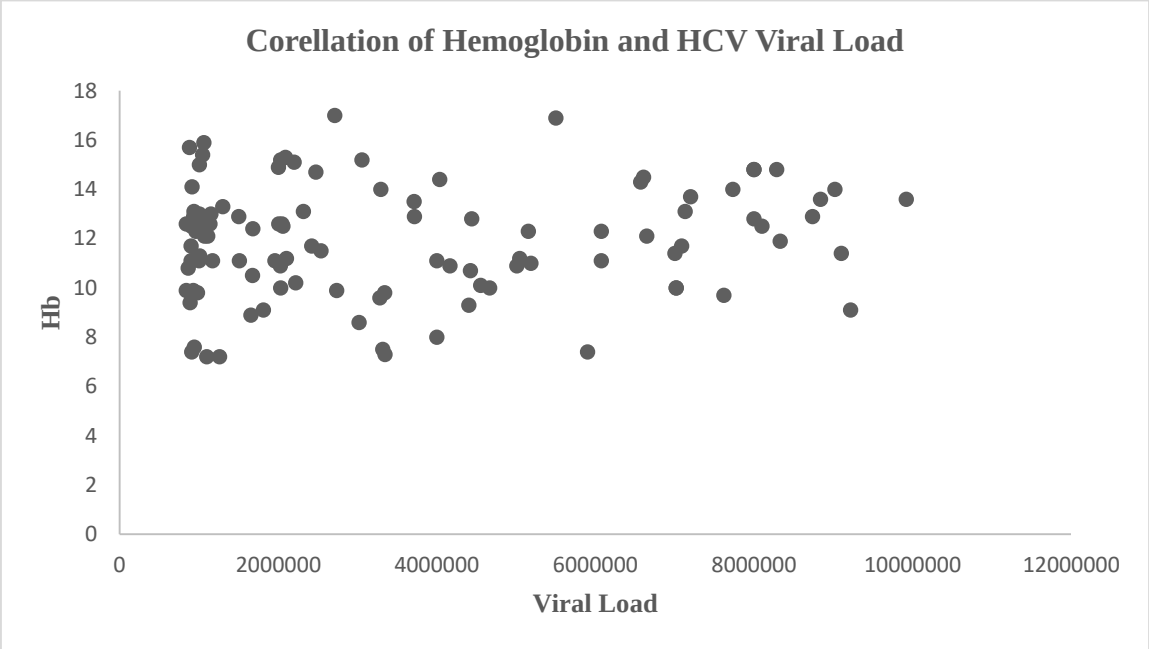


**Figure 9:** ***Correlation of Hemoglobin (Hb) and HCV Viral Load.*** **A non-significant correlation was found between viral load and hemoglobin (r = -0.018, p = 0.857).**

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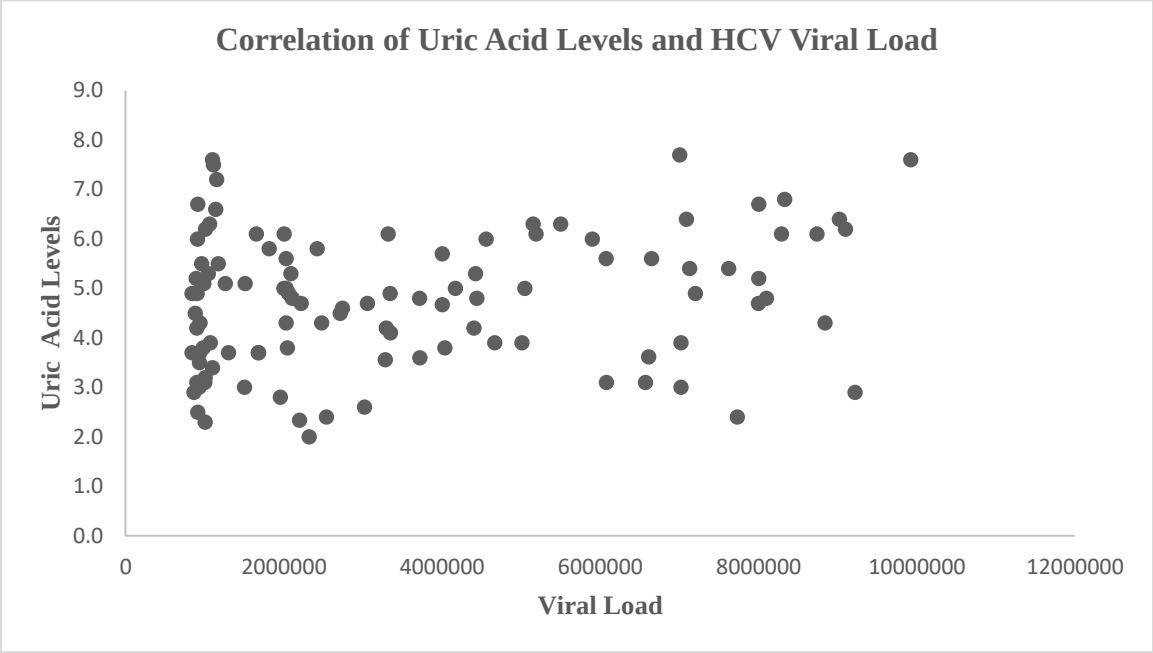


**Figure 10: *Correlation of Uric Acid Levels and HCV Viral Load*. Pearsons correlation analysis revealed a non-significant positive correlation between Viral Load and Uric Acid (r = 0.101, p = 0.304).**

Pearsons correlation shows a significant positive correlation of viral load with triglycerides levels (r = .257**, p=0.008 **fig 8**), ALT levels (r = .346**, p=<0.001 **fig 5**), and WBCs (r = .832**, p<0.001 **fig 7**). It means if the viral load increases triglyceride levels, ALT levels and WBCs will also increase. The correlation was statistically significant at the 0.01 level. Uric acid (r = 0.101, p=0.304 **fig 10**) and total cholesterol (r = .137, p=0.76 **fig 6**) shows a non-significant positive correlation. The p-value suggests that the observed correlation could have occurred by chance. However, hemoglobin (r = -0.018, p=0.857 **fig 9**) shows a non-significant negative correlation with HCV viral load. But the Pearson correlation coefficient was near zero, suggesting a weak or negligible association.

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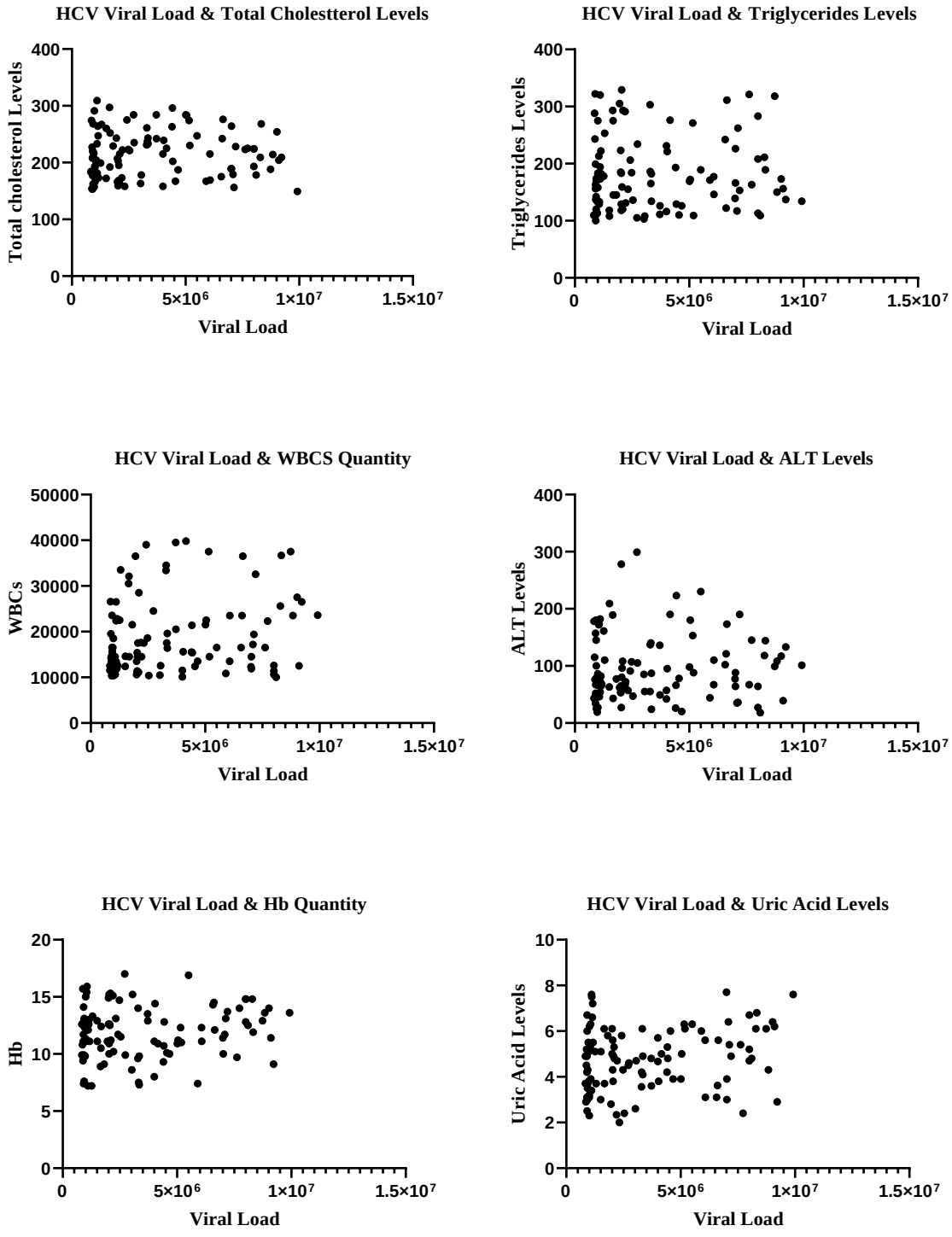


**Figure 11:** ***Correlation of HCV Viral Load with Hematological and Biochemical Parameters***

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## DISCUSSION

Pakistan is said to be one of the highest prevalence countries of hepatitis C, and the population suffering from this disease is about 6% (Umer and Iqbal, 2016). Hepatitis C infection is associated with deranged hematological, biochemical, and lipid profile parameters. If HCV infection is not diagnosed & and treated on time, it will lead to life-threatening conditions like liver cirrhosis, fibrosis, and HCC (hepatocellular carcinoma) (Chacko and Samanta, 2016).

The current study shows a significant correlation of ALT ($p<0.01$) with HCV viral load, which means if viral load increases, levels of ALT also increase. Our results are in line with (Nar and Milletli Sezgin, 2016) showing a significant correlation between viral load and ALT levels ($p= <0.01$). (Zechini et al., 2004) ) also shows a significant correlation of HCV RNA with ALT levels ($p= 0.01$). Our results are contrary with (Liu et al., 2009) that show non-significant correlations with ALT level ($r=0.40$, $P=0.695$).

In this study, triglyceride levels ($p= <0.01$) showed a significant correlation with HCV viral load, which means if the viral load increases triglyceride levels also increase. These results are consistent with (Hsu et al., 2009) showing a significant correlation between HCV viral load with triglycerides ($p= <0.05$). Our results are inconsistent with (Khattab et al., 2012) showing an inverse relation of HCV viral load with triglycerides levels.

In the current study, WBC showed a significant correlation ($p<0.01$), the results are in line with (Nar and Milletli Sezgin, 2016) showing a significant correlation of HCV RNA titers with WBCs ($p= <0.01$) which means WBCs are increased with the increase in viral load. Another study (Tsai et al., 2015) showed higher levels of WBCs in HCV patients. The current study showed a non-significant correlation for total cholesterol ($p=0.076$), an increase in HCV viral load does not affect cholesterol levels. Our results are contrary to (Hsu et al., 2009) showing a positive correlation($p= <0.05$) of HCV viral load with total cholesterol.

In this study, hemoglobin showed a non-significant correlation ($p=0.857$), which means that with an increase in HCV viral load, hemoglobin levels were not affected. Our results are in line with (Dieterich & Spivak, 2003) showing a decrease in platelets and neutrophils but no effect on hemoglobin but our result is contrary to (Chen et al., 2020) showing a decrease in hemoglobin due to interferon therapy. Another study (Chao et al., 2001) showed a HCV association with hemolytic anemia.

In the present study, uric acid showed non-significant correlation ($p=0.304$) so it means with the increase in HCV viral load, the uric acid levels did not increase nor decrease. This result is in line with another study (Jang et al., 2018), observing that there is no significant difference in uric acid levels ($p= 0.3$) between the control group and HCV patients. It means there is no decrease in uric acid levels due to HCV viral load. But our results are contrary with (Petta et al., 2012) showing a hyperuricemia in CHC patients independently.

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## CONCLUSION

The study concludes that fatigue is the most common clinical finding among HCV patients and viral load has varying degrees of association with ALT levels, total cholesterol levels, triglycerides levels, WBCs quantity, hemoglobin quantity, and uric acid levels. However, the relationships were statistically significant for ALT, triglyceride, and WBCs quantity. Additionally, there is no relationship found between viral load and hemoglobin quantity, total cholesterol levels, and uric acid levels.

### Limitations & Recommendations

The sample size of the current was small and it was a cross-sectional type of study. However, we recommend further studies with a larger sample size and cohort type of study because that will be more representative of the population.

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