

Evaluation of Hepatitis C Viral Load Between a Sample of Acute and Chronic Patients in Relation with Some Biovital and Biochemical Markers

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ABSTRACT: hepatitis C virus (HCV) infections resemble a huge global public health issue particularly in Iraq and frequently result in chronic liver disease and hepatocellular carcinoma (HCC). The major purpose of this research was to examine the viral load and liver enzymes levels in Iraqi patients with acute and chronic HCV, as well as the infection distribution by age, sex, and disease stage. Fifty patients diagnosed with HCV utilizing ELISA (Enzyme-Linked Immunosorbent Assay) for HCV antibody detection and PCR (Polymerase Chain Reaction) for HCV viral load measurement participated in this study. Based on the duration the infection lasted, the patients were categorized as either acute or chronic. Additional measures were used to evaluate the liver's function, such as the levels of ALT and AST enzymes. The viral load levels, liver enzyme values, infection distribution by sex, and age were all compared using SPSS version 27. The acute patients showed a higher mean viral load than chronic patients. In addition, the study found that the prevalence of HCV infection was highest in the 40-49 age group, and that males were more likely to have the infection (54%). The acute group and the healthy control group differed significantly only in the levels of AST liver enzymes. Acute infection was more common in younger people, and men were somewhat more impacted. The results highlight the significance of HCV monitoring at all times, since the virus can be silent or cause major liver problems.

Keywords: Hepatitis C Virus, HCV Infection in Iraq, Liver Enzymes (ALT, AST)



1. INTRODUCTION

Inflammation of the liver, or hepatitis, can be caused by several things including excessive alcohol consumption, autoimmune diseases which the body infect own cells by mistake [1], certain medications, or environmental pollutants. Viral hepatitis, the most common kind of hepatitis, is an infection with a virus.

Hepatitis C virus (HCV) is positive-sense single-stranded RNA virus classified under the genus Hepacivirus within the Flaviviridae family. The World Health Organization reports that chronic HCV infection affected at least 58 million persons globally in 2021, with an estimated 1.5 million new cases reported year [2].

Within its 9.6 kilobase pairs, the single-stranded positive-sense RNA genome of HCV contains one open reading frame (ORF), one conserved 3'-UTR, and one conserved 5'-UTR. Through its own receptors, the hepatitis C virus is able to infiltrate hepatocytes and swiftly replicate by infecting other cells through the cellular machinery [3].

Proteins that are not structural, like as p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B, are separated from the polyprotein that is encoded by the HCV open reading frame (ORF) by cellular and viral proteases. There are around 3010 amino acids in the polyprotein. In the 5' UTR and core coding region of HCV, which includes the internal ribosome entry site (IRES) that entices ribosomes to begin translation, sequence elements and secondary structures facilitate replication [4].

When the Hepatitis C virus binds to certain hepatocyte receptors—such as CD8 and LDLR—it is able to access the cells through tight junction proteins claudin1 and occludin. Persistent infection occurs because the viral genome is heterogeneous, which enables the virus to elude the host immune system [5].

2. MATERIALS AND METHODS

2.1 STUDY PARTICIPANT

This study was carried out between November 2023 and April 2024, a total of 242 suspected patients of positive HCV antibody were collected in the Hepatology and Gastroenterology Teaching Hospital in the Medical City, Baghdad - Iraq. Only fifty patients were positive for HCV after showing positive HCV RNA in their serum by polymerase chain reaction, the study will be composed of three groups, 28 acute patients group, 22 chronic patients group and 50 healthy control group. The groups were divided according to the duration of infection, if less than six months, it will be considered as acute, if more than six months, it classified as chronic infection. Ten milliliters of blood were drawn from the patients into gel tubes to clot, the tubes were left for about half an hour at room temperature to complete the clotting process. After that, centrifugation was used (4000 rounds per minute) for 15min. to separate the serum.

2.2 IMMUNOLOGICAL TEST (ENZYME-LINKED IMMUNOSORBENT ASSAY)

The "anti-HCV test" looks for antibodies to the hepatitis C virus antigen in blood samples taken from patients. The guidelines provided by CAMPmedica, Romania were followed during the patient tests. The test was carried out in accordance with the procedures provided by Fortress diagnostics, United Kingdom, for the healthy control group.

2.2.1 PROCEDURE

With the exception of the blank, 100µl of the diluent solution was applied to every well. The blank well was left unfilled after adding 10µl of the negative and positive controls to their respective wells. The pipette was used to mix the 10 microliters of sample serum into each well before the plate was incubated and sealed at 37°C for 30 minutes. The ELISA plate was washed five times and then dried it by turning it upside down on filter paper and tapped off any excess wash solution. Afterwards, all wells, with the exception of the blank well, were treated with 100µl of the enzyme conjugate. After a light shaking to combine the ingredients, the plate was placed under a cover and incubated at 37 °C for 20 minutes. A total of five washes. Except for the blank well, all of the wells were filled with 50µl of substrates A and B, and then incubated at 37°C for 10 minutes with a cover to prevent light exposure. A change in color from blue to yellow was observed after adding 50µl of stop solution to the wells, which indicated good responses. A 450 nm absorbance was recorded in less than 10 minutes using an ELISA reader.

To determine the Cut-Off value, the following formula was applied: Cut-Off value (C.O.) = NCx + 0.12

*NCx = Mean OD of three negative control. * Negative Results: Sample OD < C.O. * Positive Results: Sample OD ≥ C.O.

2.3 MOLECULAR DETECTION OF HCV

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, v2.0 can detect HCV RNA using a sensitive molecular technique; it can identify both acute and chronic HCV infections. In order to confirm that the infection is active, the quantitative test checks specimens for HCV RNA. This highly sensitive test reliably identifies HCV genotypes 1–6 using fully automated sample extraction in conjunction with real-time PCR amplification and detection. Automated specimen preparation, reverse transcription, PCR amplification, and detection are all part of the COBAS AmpliPrep/COBAS TaqMan HCV Test's ability to detect HCV target RNA and HCV Quantitation Standard (QS) Armoured RNA. The Master Mix reagent contains both HCV RNA and HCV QS Armoured RNA-specific primers and probes.

One 60-second cycle at 95°C followed by 45 cycles at 95°C for 15 seconds, 55°C for 30 seconds, and 72°C for 15 seconds made up the RT-PCR protocol.

2.4 BIOCHEMICAL TESTS

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured for every patient and healthy control using automated Cobas® C111 by Roche®. The Cobas c111 measures ALT and AST based on specific enzymatic reactions. ALT and AST are enzymes that catalyze reactions involving the transfer of amino groups between amino acids and α-keto acids. The reactions catalyzed by ALT and AST are often coupled with secondary reactions involving NADH (Nicotinamide adenine dinucleotide + hydrogen) oxidation or reduction. The Cobas c111 monitors the decrease in absorbance of NADH at a wavelength of 340 nm. This decrease is proportional to the concentration of ALT or AST in the sample. The rate of change in absorbance is used to calculate the enzyme activity, which is reported in units per liter (U/L). This process allows for accurate and automated measurement of ALT and AST, which are critical indicators of liver function. The normal value was between 0-40 U/L, any value above 40 U/L is considered positive.

2.5 STATISTICAL ANALYSIS

A one-way analysis of variance ANOVA was used to determine if group variance was significant. The statistical program for social Sciences (SPSS) version 27 [6] was used to determine statistical significance and to compare the

data between groups. The data were expressed as Mean $\pm$ standard error between the numerous parameters employed in this investigation, the probability (P-value) was set at the significant level of ($P \leq 0.05$ and $P \leq 0.01$). In addition, the mean results for each group were compared using Duncan's test in SPSS statistics to check if there is a significant difference.

3. RESULTS AND DISCUSSION

In the current investigation, which involved 50 HCV patients and 50 healthy controls, we found a power analysis of 0.95, an effect size of 0.8, and an α probability value (two-tailed probability) of 0.02 for the groups under consideration, as shown in Table (1).

Table 1. - Power analysis report

Parameter	Value
Effect Size (Cohen's d)	0.8
Power (1 - β error probability)	0.95
Sample Size (Group 1)	50
Sample Size (Group 2)	50
Non-centrality Parameter	4.0000000
Critical t-value	2.3382850
Degrees of Freedom (Df)	98
Alpha Error Probability (α)	0.0214046

3.1 DETECTION OF HCV RNA

The viral load test verifies the existence of the virus infection, provides a measurable indication of illness, and aids in monitoring the treatment's efficacy, all this is done by polymerase chain reaction technique since it is the most reliable method, not time-consuming and do not show any false positive or false negative results [7].

The patients were divided into acute group and chronic group according to the time of infection. If the time of infection was less than 6 months, it considered acute, if more than 6 so it considered chronic.

The maximum and minimum viral load was (7630000 – 104) IU. The mean of viral load for the acute group was 1416591.11 and the mean of viral load for the chronic group was 782960.77, which means the acute was higher than the chronic group but, statistically there are no significant difference between them whether in males or females. The results are illustrated in table (2).

Table 2. - Viral load levels between acute and chronic patients

Sex	Viral load mean $\pm$ SE (Unit)		Probability
	Acute	Chronic	
Total	1416591.11 $\pm$ 381263.86	782960.77 $\pm$ 528366.39	0.198
Males	1319118.57 $\pm$ 455291.90	604724.77 $\pm$ 232222.34	0.184
Females	1514063.64 $\pm$ 628537.63	1040412.78 $\pm$ 528366.39	0.602
Probability	0.804	0.410	

A positive HCV viral load is not necessarily indicative of liver damage, as several studies have shown, as the viral load is evaluated in the blood, not the liver. No previous study has linked elevated blood HCV RNA levels to an increased risk of HCC [8]. But, according to a study done in 2016 by Noh et al. [9], those with a high HCV RNA titer ($\log$ HCV RNA IU/mL > 6) had an incidence rate of 474.1 per 10,000 person-years for HCC, while those with a low titer ($\log$ HCV RNA IU/mL ≤ 6), had an incidence rate of 111.5 per 10,000 person-years ($P=0.032$).

The patients' normal ALT and AST values, among others, are explained by the fact that earlier studies have shown no link between any of the clinical and laboratory markers and HCV viral loads [10]. Similarly, the severity of liver disease is not dependent on blood levels of hepatitis C virus. Other study by Schijman et al., which included 396 individuals with chronic infections from India, Russia, Uruguay, and Argentina, is in agreement with our study [11].

Additionally, he discovered that there is no correlation between sex and HCV RNA levels in patients, regardless of whether they were infected with various genotypes.

3.2 DISTRIBUTION OF INFECTION ACCORDING TO SEX

After examination more than 242 suspected with HCV, only 50 patients appeared to be infected with hepatitis C virus. Those patients were examined by polymerase chain reaction to ensure their infectivity. Our study revealed that the males (27) are more infected (54%) than females (23) which equals about (46%), as shown in figure (1), and that may be due to more exposing to contaminate blood transfusion through accidents or shaving or other possible causes.

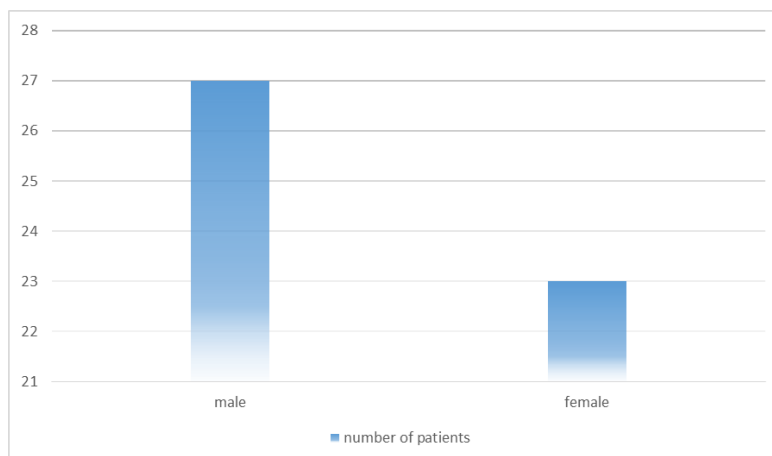


FIGURE 1. - Sex Groups Distribution for Patients Infected with HCV

Our study agrees with the most of studies in Iraq like Khudhair et al. [12] which showed the males are more likely be infected than females. Other studies like Al-Bayaa et al., [13] and Tarky et al., [14] further demonstrated that hepatitis C virus infections among individuals screened at Iraqi government health care facilities in 2018, particularly in the cities of Baghdad, Rasafa, and Sulaimaniyah, are more prevalent in males than in females.

Other study like Merzah et al., [15] disagree with us, which examined all types of hepatitis patients from 2007 to 2016 in Karbala city, the results of HCV patients showed that the females are more likely to be infected than males.

A review article was published by using meta-analysis which they calculate the total HCV patients in Egypt, which considered the most infected country with HCV worldwide. The study calculates the included studies from 2011 to 2021 with 193,621 patients with HCV. After analysis, the male patients were higher than female patients with 50.5% [16]. Many studies proved that males are more affected with HCV disease, the reason for this difference are multifactorial and include combination of biological, behavioral, and socio-economic factors. For example, sex hormones in females plays a huge role in immune response and liver disease since the liver contain many receptors for these hormones, like estrogen which may have a protective effect against liver damage [17], so that one of the reasons that explain why females are less likely to be affected by HCV. Other factors like behavioral risks like drug-use or might be less likely to seek medical care or be tested for HCV, leading to underdiagnosed and delayed treatment.

3.3 DISTRIBUTION OF INFECTION ACCORDING TO AGE

The patients were divided into six groups according to age, this groups ranged from lower than 20 year to upper than 60 year, the results revealed that most of the patients were located in the fourth group ranged between 40-49 years (24%), other groups were ranged: group (a) less than 20 years old only two patients (4%), group (b) from 20 to 29 years old only 9 patients (18%), group (c) from 30 to 39 years old only 11 patients (22%), group (d) as mentioned above only 12 patients (24%), group (e) ranged from 50 to 59 years old only 9 patients and lastly group (f) which show only 7 patients more than 60 years old as showed in the figure (2).

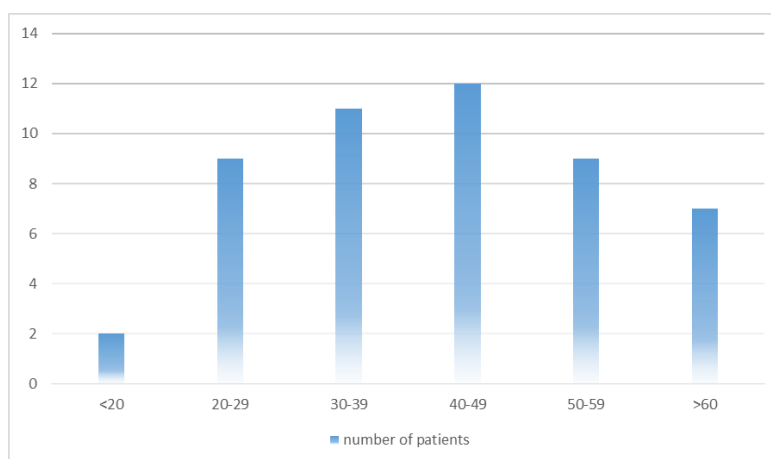


FIGURE 2. - Age Groups Distribution for Patients Infected with HCV (y-axis resembles number of patients, x-axis resembles age)

Our study divided the results into three groups acute, chronic and healthy controls as shown in table (3), statistical analysis also showed there are no significant correlation between age and sex groups as shown in table (4).

Table 3. - Viral load levels between acute and chronic patients

Groups	Age mean $\pm$ SE (Years)
Control	39.76 $\pm$ 1.11 a
Acute	39.54 $\pm$ 2.87 a
Chronic	44.59 $\pm$ 2.33 a

(Duncan's test: similar letters mean there are no significant differences between groups).

Table 4. - Distribution of HCV patients, healthy control groups according to their sex groups.

Groups	Age mean $\pm$ SE (Years)		Probability
	Males	Females	
Control	39.89 $\pm$ 1.57 a	39.59 $\pm$ 1.60 a	0.924
Acute	40.21 $\pm$ 3.33 a	38.86 $\pm$ 4.80 a	0.748
Chronic	46.23 $\pm$ 2.46 a	42.22 $\pm$ 4.53 a	0.409

(Duncan's test: similar letters mean there are no significant differences between groups).

The findings of this results indicate the age group 20-29 had highest number of patients with acute infection, while the age group 40-49 years had higher number of chronic patients. Several factors may contribute to this results especially for younger patients like high-risk behaviors, tattoo, drug use, etc.

For chronic patients the possible explanation is may be due delayed diagnosis which remained undiagnosed for years, contaminated blood transfusion or medical procedures.

Khudhair *et al.* (2020) [12] found that the prevalence of HCV infection peaked in the 1–20 group, then dropped to 21–40, and 41–60 groups in after that. However, the poor medical care in Thiqr may explain why the infection prevalence was lowest in the >60 age group.

Another study in 2018 which examined HCV patients in Governmental Health Care Facilities in Iraq and divided the HCV patients into five groups and showed the most effected group was between the ages 15-45 years old which was 343 of total 546 patients in different cities in Iraq but mostly in Baghdad with 123 patients [13].

Other study proved the HCV infection is more common in the age above 40 years old by examining more than 9000 suspected with HCV from the duration January 2005 to December 2006 in Iraq [14].

Al-Mussa & Thamair [18] published a study in 2019, which they examined 162137 suspected with HCV in Basrah-Iraq, only 355 were shown to be infected with the hepatitis C virus and the patients ranged from 15-45 years old.

In 2023, Lafta *et al.* [19] examined 50 patients in the Gastroenterology and Hepatology Teaching Hospital in Baghdad, Iraq's Medical City, he found that the age group from 20-29 years old had the highest frequency of HCV

infection at 23 patients, while the age groups from 30-39 years old had the lowest frequency at 1 patient and from ≥ 50 years old at 1 patient each. Virus genotypes might play a role in this distribution, according to a 2013 study by Petruzzello *et al.* [20]. Consistent with prior medical practice, the study indicated that subtypes 1b and 2a/2c were more prevalent in persons older than 60 years old. Subtypes 3 and 4 were more prevalent, however, among those aged 31 to 60.

The rapid evolution of HCV makes it more challenging for the immune system to establish a durable and efficient defense. Although some people manage to get over the virus on their own after getting infected, many others don't build up enough immunity, which leaves them vulnerable to persistent infections. Immune systems also tend to get weaker with age. Chronic infection is more common in the elderly since this can lower the immune system's ability to fight off infections, including HCV.

3.4 DISTRIBUTION OF PATIENTS ACCORDING TO STAGE OF DISEASE

From the 50 positive patients that had been infected with HCV, 28 patients diagnosed with acute HCV (56%) that have been infected with HCV in less than 6 months. Those patients were newly diagnosed which had been exposed to an accident (syringes, contaminated blood transfusion, etc.) less than 6 months in addition to appearing of symptoms like fatigue, pain in abdomen, jaundice and other symptoms which tested positive to HCV RNA.

In contrast, 22 patients (44%) developed chronic illness due to a prior infection that lasted longer than 6 months. Prolonged and untreated chronic hepatitis C can cause fibrosis, cirrhosis, and hepatocellular carcinoma, among other significant complications. Many people with long-term infections don't have any symptoms until the liver scars (cirrhosis). The results are illustrated in figure (3).

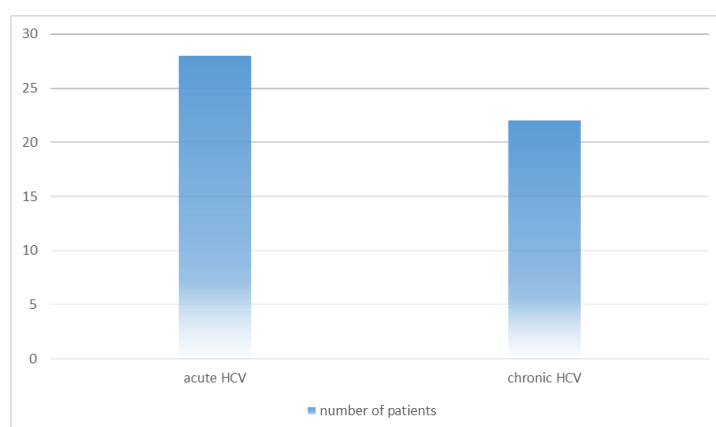


FIGURE 3. - Number of acute and chronic patients.

The number of acute and chronic patients that divided into groups by the age are illustrated in table (5).

Table 5. - Viral load levels between acute and chronic patients.

Sex No. (%)	Groups		
	Control	Acute	Chronic
Males	28 (56.0)	14 (50.0)	13 (59.1)
Females	22 (44.0)	14 (50.0)	9 (40.9)
Total	50 (100.0)	28 (100.0)	22 (100.0)

The results indicated a nearly equal proportion of male and female patients in both acute and chronic groups, though there was a slightly higher percentage of chronic HCV in males (59.1% vs. 40.9% in females). This suggests that while both sexes are equally affected by acute HCV, males may be at a higher risk for progression to chronic infection. The acute and chronic patients in addition to the controls are grouped according to age, as shown in table (6).

Table 6. - Number of acute, chronic patients and healthy control group according to age

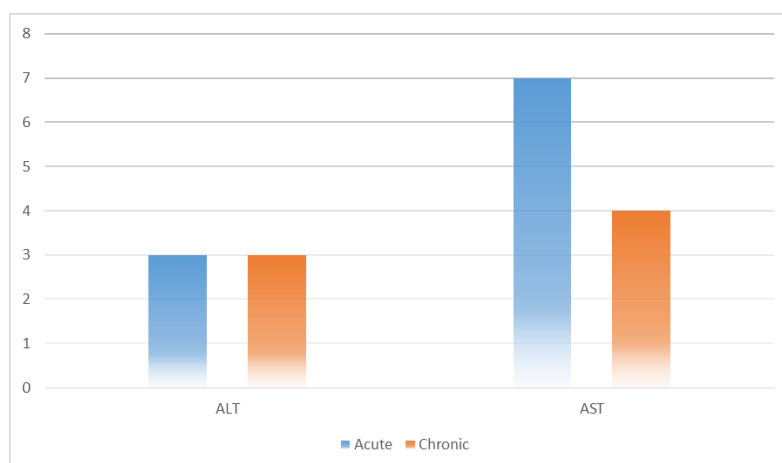
Age groups No. (%)	Groups		
	Control	Acute	Chronic
Less than 20	0 (0%)	2 (7.14%)	0 (0%)
20 – 29 years	11 (22%)	7 (25%)	2 (9.09%)
30 – 39 years	17 (34%)	6 (21.43%)	5 (22.73%)
40 – 49 years	15 (30%)	3 (10.71%)	9 (40.91%)
50 – 59 years	6 (12%)	5 (17.86%)	4 (18.18%)
More than 60	1 (2%)	5 (17.86%)	2 (9.09%)
Total	50 (100%)	28 (100%)	22 (100%)

The highest proportion of acute cases (25%) occurred in the 20–29-year age group, whereas the 40–49-year age group had the highest proportion of chronic cases (40.91%). This finding suggests that younger individuals may be more likely to contract HCV acutely, potentially due to lifestyle factors such as higher rates of exposure to risk factors like injecting drug use. Older patients, particularly those in the 40–49-year range, may experience a longer delay in diagnosis, or low immune response, allowing the infection to progress to a chronic stage.

3.5 BIOCHEMICAL TEST RESULTS

The results of the liver enzymes ALT and AST were divided into groups, acute and chronic. For ALT enzyme (normal range is less than 40 U/L), it was found that in the acute group it contains 3 of 28 patients that have abnormal values (10.7%). For the chronic group, it was found that 3 of 22 patients have abnormal value (13.6%).

For AST enzyme (normal range less than 40 U/L), it was found that in the acute group it contains 7 of 28 patients that have abnormal values (25%). For the chronic group, it was found that 4 of 22 patients have abnormal value (18.1%) as shown in Fig. (4).

**FIGURE 4. - Number of patients that have abnormal values of ALT & AST**

For alanine transaminase (ALT) enzyme the values showed there were no significant difference between healthy controls and the acute and chronic groups in both sexes since it has similar letters in Duncan's test and the P value were 0.470 for the control group, 0.529 for the acute group and 0.635 for the chronic group, as shown in table (7).

Table 7. - Values of ALT enzyme among the study groups

Groups	ALT level mean $\pm$ SE (mg/dl)			Probability
	Total	Males	Females	
Control	14.79 $\pm$ 1.97 a	17.10 $\pm$ 1.65 a	23.22 $\pm$ 3.88 a	0.260
Acute	25.57 $\pm$ 4.49 a	22.66 $\pm$ 4.58 a	28.47 $\pm$ 7.85 a	0.529
Chronic	20.78 $\pm$ 4.62 a	18.88 $\pm$ 3.88 a	23.49 $\pm$ 10.13 a	0.635

(Duncan's test: similar letters mean there are no significant differences between groups)

For aspartate aminotransferase (AST) enzyme the values showed there were significant difference between healthy controls and the acute group and in female sex particularly. The chronic group showed there was no significant difference between the studied groups. The p values when compared between sexes were 0.611 for the control group, 0.219 for the acute group and 0.716 for the chronic group. The results shows that the acute group have higher mean value than the two other groups which the mean $\pm$ SE was 38.08 $\pm$ 9.65 as shown in table (8).

Table 8. - Values of AST enzyme among the study groups

Groups	AST level mean $\pm$ SE (mg/dl)			Probability
	Total	Males	Females	
Control	16.44 $\pm$ 1.28 b	14.76 $\pm$ 0.92 a	18.57 $\pm$ 2.63 b	0.642
Acute	38.08 $\pm$ 9.65 a	26.06 $\pm$ 3.81 a	50.09 $\pm$ 18.71 a	0.219
Chronic	24.31 $\pm$ 3.99 ab	23.06 $\pm$ 4.49 a	26.12 $\pm$ 7.60 ab	0.716

(Duncan's test: similar letters mean there are no significant differences between groups)

ALT and AST levels can be normal in patients with Hepatitis C Virus (HCV) infection. While elevated levels of these liver enzymes are often associated with liver inflammation or damage, HCV infection doesn't always lead to noticeable changes in these enzyme levels. Many studies proved the liver enzymes can be normal like the study of Zapata [21], which said HCV patients can have normal alanine aminotransferase levels but it can be elevated and become abnormal with time.

In 2004, Ahmed and Keefe [22], demonstrated that "the inability to identify infected patients when they are asymptomatic and have normal ALT levels." Their results are consistent with ours. When trying to put together the evolution of chronic hepatitis C.

In order to test the idea that patients infected with HCV experience levels of ALT that fluctuate over time, a study [23] were done to measure serial serum ALT levels four times for 25 months. When 647 HCV patients were examined for the first time, normal ALT levels were found. However, 323 had an increased ALT of four measures in the 25 months that followed.

We also agree with a study that measures the liver enzyme between different genotypes in northern of Iraq, which revealed there weren't a significant differences between liver enzymes ALT and genotype 4, which it's the most prevalent genotype in Iraq, but we disagree when they found normal AST values with the same genotype [24, 25].

A similar study in Iraq, which examined the liver enzymes ALT and AST on 90 patients, 70 patients seem to have normal liver enzymes while the patients infected with hepatitis C virus [26].

Another study in 2004 by Zechini et al. [27] which supports our study by examining 112 patients with chronic hepatitis C virus. The study examined ALT and AST of the patients and they were followed up for 6 months, the results were observed is normal ALT and AST at the baseline and there were no significant difference between chronic patients and healthy controls but the enzymes were elevated when measured after several months.

We disagree with the study by Mohammed et al. [28] when they studied the Serum Hepcidin Level in Iraqi Patients with Chronic Hepatitis C, they found there was a significant difference after measurement of liver enzymes ALT, AST, ALP and TSB.

Another study by Al-Bayati et al. [29] who published research in 2023 when examined chronic hemodialysis Patients in Kirkuk City-Iraq. Although the study did not mention whether the patients were acute or chronic, the study founds levels of ALT, AST, ALP, and IL-6 were statistically significant between the patients and healthy control groups.

Abnormal liver enzymes are not always seen in HCV patients, according to several studies. The enzyme ALT aids in the conversion of proteins into energy that the liver cells can use. The release of ALT into the bloodstream and

subsequent elevation of levels occurs in response to liver injury. Amino acid synthase (AST) is an essential enzyme for protein synthesis. Blood levels of AST are typically modest, similar to those of ALT. A rise in AST levels could indicate injury to the liver, disease of the liver, or harm to the muscles. Even in healthy individuals, a normal ALT level could be an indicator of serious liver disease [30].

4. CONCLUSION

This study examined viral load, age, sex distribution, and liver enzyme levels (ALT, AST) in 50 HCV patients across acute and chronic stages. While the acute group had a higher viral load, no significant difference was found between groups. Male HCV patients were more than female patients, possibly due to behavioral and socioeconomic factors. Acute cases peaked in ages 20–29, while chronic cases were highest in 40–49. Only AST showed significant differences between acute and control groups, emphasizing the need for continuous monitoring regardless of liver enzyme levels.

5. Ethical approval

The study was approved by the Ethics Committee of the College of Science/Mustansiriyah University (Ref.: BCSMU/1221/00039M). All study subjects provided informed consent.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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