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Is the Hepatitis G Virus a Hidden Menace to Liver Health in Specific Populations?

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ABSTRACT

Background: Hepatitis G virus (HGV) infection is widespread worldwide in high-risk groups such as healthcare workers, voluntary blood donors, and chronic liver disease patients. Also, haemophilia and thalassemia are diseases within the high-risk groups for HGV infection. Although it has no clinical significance or impact on liver disease and its future course is uncertain, highly mutated (as in other RNA viruses) might happen, resulting in a severe liver injury.
Objectives: To evaluate the subclinical impact of hepatitis G virus infection on liver health in high-risk populations through liver function tests (LFTs) analysis.
Materials and methods: Blood samples were taken from 221 people in high-risk groups and chronic liver patients infected with hepatitis B and C viruses. An enzyme-linked immunosorbent assay (ELISA) was used to find HGV anti-E2 antibodies, and a multiplex nested real-time polymerase chain reaction method was used to find HGV RNA. LFTs were performed for all participants, which included checking the levels of AST, ALT, total bilirubin, AP, and serum albumin. A self-controlled case study was used to look at differences in liver function tests between people with a high risk and people who have chronic hepatitis B and C with or without HGV infection.
Results: HGV infection was more prevalent in high-risk groups like patients with chronic liver disease (15.8%), healthcare professionals (6.8%), haemophiliacs (6.8%), and thalassemia patients (9.5%) compared to volunteer blood donors (3.2%) and recipients of blood (3.6%). There was no statistically significant difference (P-value $>$ 0.05) in the abnormal rates of ALT, AST, serum albumin, ALP, and total bilirubin between high-risk groups and chronic liver patients regardless of whether they had HGV infection or not.
Conclusion: Surveillance for HGV infection does not appear to cause significant liver dysfunction in high-risk and chronic liver patients.
Keywords: Hepatitis G virus; Liver function tests; Haemophilia; Thalassemia; Chronic viral hepatitis.

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INTRODUCTION

Viral hepatitis is an infection that causes liver inflammation and damage. There are several types of hepatitis viruses, including hepatitis A, B, C, D, and E [1]. Hepatitis G virus (HGV), also known as Human pegivirus (HPgV-1) or GB virus C (GBV-C), is a single-stranded ribonucleic acid (RNA) virus that is related to hepatitis C virus (HCV) [1, 2]. It shares some genetic similarities with HCV and is found in patients with chronic liver disease [3, 4]. However, unlike other hepatitis viruses, its role in hepatitis disease is still unclear [1, 3]. HGV is typically spread through blood and blood products, intravenous drug use, and other activities that carry a high risk of parenteral blood exposure [5, 6].

HGV is common worldwide among high-risk groups, including people with hemophilia, and thalassemia, healthcare workers, blood donors, and people with chronic liver disease. However, HGV infection has minimal clinical consequences and little impact on liver health. The natural history of HGV

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infection and its possible role in disease progression remains unclear [7].

HGV causes no noticeable symptoms or signs of liver disease [3, 5]. However, in some cases of chronic or long-lasting HGV infection, its presence in the bloodstream (viremia) has been documented [4, 5].

HGV infection rates are high among high-risk groups and blood donors in Taiwan. However, the exact clinical implications and role of HGV in liver disease progression are still not determined in those populations [1, 7]. Enzyme-linked immunosorbent assay (ELISA) detection test HGV RNA alone is not enough to fully check the effects of the virus on the liver [8]. Additional diagnostic tests, such as assessment of liver function tests (LFTs), are needed to confirm the clinical effects of HGV and evaluate its influence on liver health and damage [4]. Measuring liver enzyme levels can help determine the effects of an HGV infection on liver function [3, 9].

Owing to the low virulence of the HGV, little research has been conducted on the prevalence and its clinical impact in hepatitis B and C patients with chronic liver disease and high-risk populations. However, HGV, like other RNA viruses such as HCV and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can become more aggressive after rapid and significant mutations [10, 11]. During the coronavirus disease 2019 (COVID-19) pandemic, we have seen that the causative virus SARS-CoV-2 can cause systemic manifestations (such as cough, shortness of breath, smell and taste disorders, etc.), aggressive morbidities such as liver dysfunction, rhino-orbital cerebral mucormycosis, and even mortality [12, 13]. Therefore, this study was done to find out if the normality rates of AST (aspartate aminotransferase), ALT (alanine transaminase), ALP (alkaline phosphatase), total bilirubin, and serum albumin were different in high-risk people who had or did not have HGV infection.

MATERIALS AND METHODS

In the current prospective comparative study, 86 individuals were excluded from the original 307 participants (Figure 1). The prevalence of the Hepatitis G virus in the general population of Asia was 12.0%, while the prevalence among high-risk groups was 30.0% [3]. Therefore, the Kelsey equation in Epi Info version 7.2 was used to calculate a sample size of 159 with a 95% confidence interval. However, we enrolled 221 patients during the designed period of the current study.

Between September 22, 2022, and April 12, 2023, the participants were interviewed at five medical facilities in Baghdad and Anbar Province, Iraq (Al Karama Teaching Hospital, Ramadi Teaching Hospital, Ramadi Teaching Hospital for Maternity and Children, Anbar Blood Transfusion Center, and Teaching Hospital for Digestive and Haematology. Informed consent was obtained from all participants. The Ethical Approval Committee of the University of Anbar in Iraq gave its approval to the study (Reference number 130, on 11-12-2023).

Blood samples were collected from all 221 participants to detect HGV anti-E2 antibodies using the ELISA technique (LifeSpan BioSciences USA)), and the presence of HGV RNA using a multiplex nested real time-polymerase chain reaction (RT-PCR) method (Applied Biosystems, USA). The primers used were forward: GCCCTACCGGTGGAATAAG and Reverse: CCCACTGGTCCTTGTCAACT.

LFTs were performed for all participants, which included checking the levels of AST, ALT, total bilirubin, ALP, and serum albumin. A self-controlled case study design was conducted to compare abnormalities in LFTs between high-risk individuals and chronic hepatitis B and C liver patients with and without HGV infection (reference blood test results for typical LFTs as the follows: ALT = 7 to 55 units per litre (U/L), AST = 8 to 48 U/L, ALP = 40 to 129 U/L, Albumin = 3.5 to 5.0 grams per decilitre (g/dL), and bilirubin = 0.1 to 1.2 milligrams per decilitre (mg/dL). The results of LFTs for people who tested positive for HGV infection were compared with the results of the same people without infection.

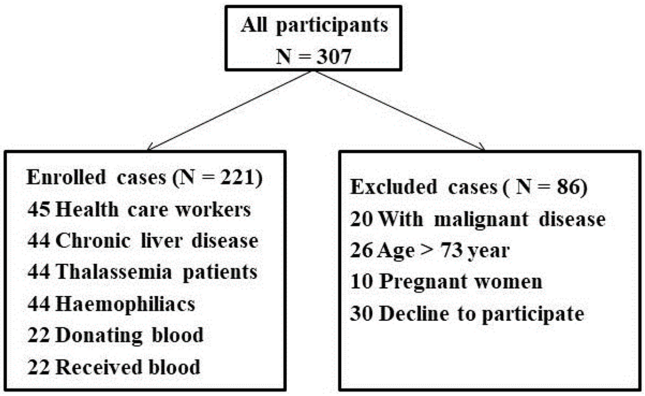

Figure 1. Flow chart of the 307 participants.

Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 26.0 and GraphPad Prism. Descriptive statistics including mean and standard deviation were calculated for continuous variables. Independent sample *t*-test and one-way ANOVA were used to compare liver function enzyme levels between high-risk subjects and chronic hepatitis B and C patients with or without HGV infection. Appropriate statistical tests such as cross-tabulations and Chi-square tests were used to determine any differences in LFTs abnormalities between groups with and without HGV co-infection. The results were presented using figures or tables to facilitate interpretation and understanding of the comparisons between groups. A P-value of < 0.05 was considered a statistically significant difference.

RESULTS

Participants who tested positive for anti-E2 antibodies had a higher mean age than those who tested negative (33.61 $\pm$ 14.90 years and 30.20 $\pm$ 15.40 years respectively). However, it was no statistically significant difference (P- value = 0.617). Besides, there was no significant difference (P-value = 0.162) in the mean age of female and male participants who tested positive for anti-E2 (34.03 $\pm$ 16.3 years and 33.4 $\pm$ 14.2 years, respectively), as shown in Table 1.

Frequency of HGV in different cases

Of the total 221 participants, anti-E2 HGV antibodies using the ELISA test were positive in 35 of 44 (15.8%) patients with chronic liver disease, 7 of 22 (3.2%) healthy blood donors, 15 of 45 (6.8%) healthcare workers, 15 of 44 (6.8%) haemophiliacs, 8 of 22 (3.6%) blood recipients, and 21 of 44 (9.5%) thalassemia patients. While HGV RNA was positive in 34 of 44 (15.4%) patients diagnosed with chronic liver disease, 7 of 22 (3.2%) healthy blood donors, 15 of 45 (6.8%) healthcare workers, 14 of 44 (6.3%) haemophiliacs, 7 of 22 (3.2%) blood recip-

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Table 1. Mean age of participants according to HGV positivity and gender. ELISA = enzyme-linked immunosorbent assay test.

ELISA result		N	Mean age per year	Std. Deviation	P- value
Positivity	Negative	120	30.20	15.40	0.617
	Positive	101	33.61	14.90	
Gender					
Negative	Female	36	27.17	15.816	0.162
	Male	84	31.46	15.130	
Positive	Female	35	34.03	16.30	
	Male	66	33.40	14.20	

ients, and 20 of 44 (9.0%) thalassemia patients. The prevalence of HGV infection was significantly higher in patients with chronic liver disease, healthcare workers, haemophilia patients, and thalassemia patients than in healthy volunteer blood donors and recipients (P-value = 0.001) as shown in Figures 2.

Normality rate of ALT in participants with or without HGV using the ELISA test

Of 101 participants who tested positive for anti-E2 HGV antibodies using an ELISA test, 17 (7.7%) had abnormal ALT levels. Of the 120 participants who tested negative for HGV anti-E2 antibodies, 5.9% (13 participants) had abnormal ALT levels. There was no statistically significant difference in ALT abnormality rates between high-risk groups and chronic liver patients, regardless of whether they had HGV infection (P-value = 0.195) as shown in Figure 3.

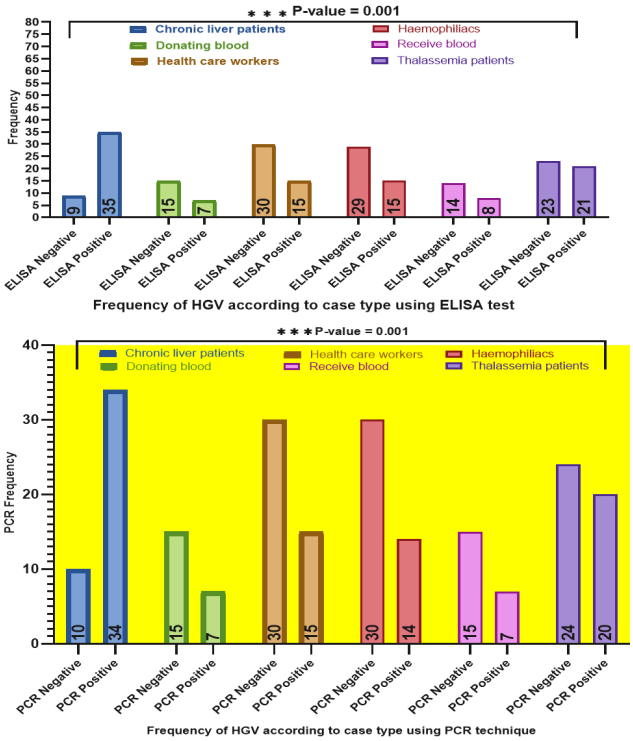

Figure 2. Frequency of the hepatitis G virus according to case type using the polymerase chain reaction (PCR) test.

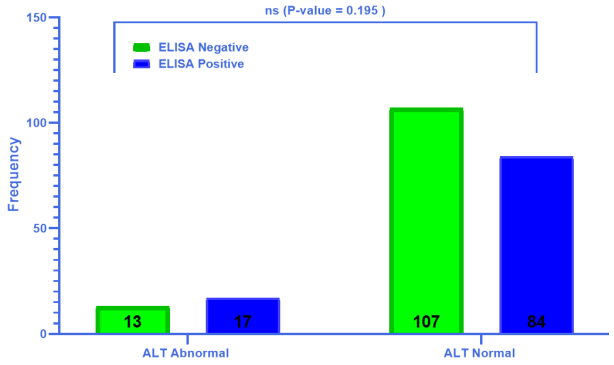

Figure 3. Normality rate of ALT in participants with or without Hepatitis G virus using an enzyme-linked immunosorbent assay (ELISA) test.

Normality rate of AST in participants with or without HGV

Of 101 participants who tested positive for anti-E2 HGV antibodies using an ELISA test, 29 (13.1%) had abnormalities in AST levels. In contrast, 13.5% (30 participants) of the 120 participants who tested negative for HGV anti-E2 antibodies had abnormal AST values. There was no statistically significant difference in AST abnormality rates between high-risk groups and chronic liver patients regardless of whether they were infected with HGV or not (P- value = 0.434) as indicated in Figure 4.

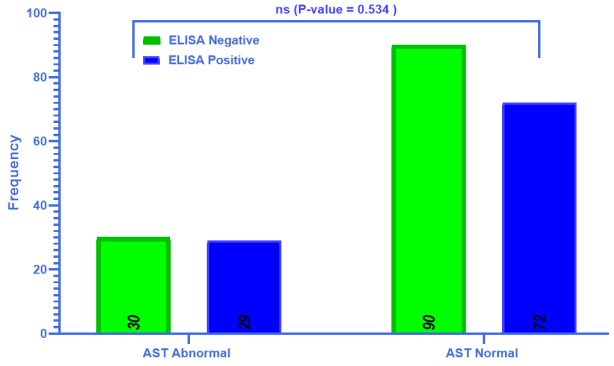

Figure 4. Normality rate of AST in participants with or without Hepatitis G virus using enzyme-linked immunosorbent assay (ELISA) test.

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Table 2. Normality rate of serum albumin in participants with or without Hepatitis G virus using enzyme-linked immunosorbent assay (ELISA) test.

Serum albumin normality	ELISA Result		Total	P- value
	Negative	Positive		
Abnormal	1 (0.5%)	3 (1.4%)	4 (1.8%)	
Normal	119 (53.8%)	98 (44.3%)	217 (98.2%)	0.235
Total	120 (54.3%)	101(45.7%)	221 (100.0%)	

Normality of serum albumin in participants with or without HGV

Of the 101 participants who tested positive for HGV anti-E2 antibodies by ELISA, 3 (1.4%) had abnormal serum albumin levels. In contrast, only 0.5% (1 participant) of the 120 participants who tested negative for HGV anti-E2 antibodies had abnormal serum albumin levels. There was no statistically significant difference (P-value = 0.235) in the rates of serum albumin abnormalities between the two groups, which comprised people with high risk and people with chronic liver disease, with or without HGV infection (Table 2).

Normality of ALP in participants with or without HGV

Of the 101 participants who tested positive for HGV anti-E2 antibodies by ELISA, 38 (17.2%) had abnormal ALP levels. In contrast, 21.3% (47) of the 120 individuals who tested negative for HGV anti-E2 antibodies had abnormal ALP levels. There was no statistically significant difference (P-value = 0.814) in ALP abnormality rates between the two groups (Figure 5).

Normality of total bilirubin in the participants with or without HGV

Of the 101 participants who tested positive for HGV anti-E2 antibodies by ELISA, 73 (33.0%) had abnormal total bilirubin levels. In contrast, 38.7% (81 participants) of the 120 people who tested negative for HGV anti-E2 antibodies had abnormal total bilirubin levels. There was no statistically significant difference (P-value = 0.268) in total bilirubin abnormality rates between the two groups (Figure 6).

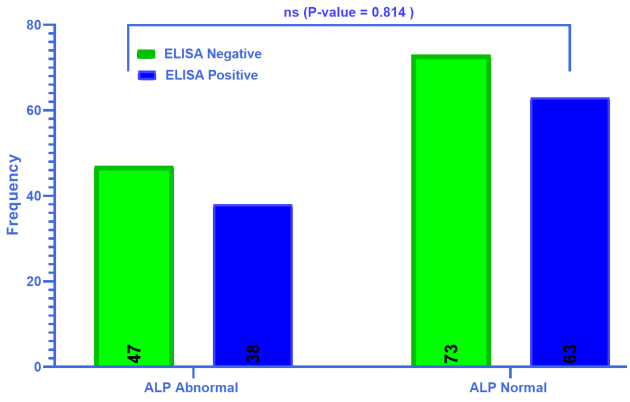

Figure 5. Normality rate of ALP in the participants with or without Hepatitis G virus using enzyme-linked immunosorbent assay (ELISA) test.

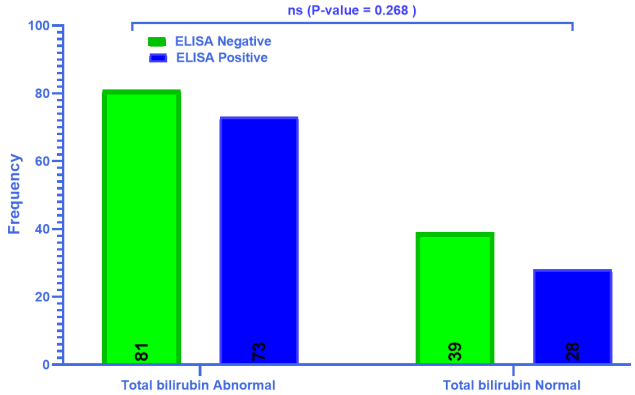

Figure 6. Normality rate of total bilirubin in the participants with or without Hepatitis G virus using enzyme-linked immunosorbent assay (ELISA) test.

DISCUSSION

HGV, a type of hepatitis virus, is a single-stranded RNA virus that is transmitted through blood. According to a study of 67,348 blood donors, the prevalence rate among blood donors in mainland China is about 4% [3]. In most cases, infection with the hepatitis G virus is mild, often asymptomatic, or has few symptoms at the time of infection. Like the HGV, RNA viruses such as the hepatitis C virus and SARS-CoV-2 can also become aggressive after rapid and specific mutations. During the COVID-19 pandemic, we have seen that the causative virus (SARS-CoV-2) can cause systemic and severe manifestations (e.g., cough, shortness of breath, loss of smell and taste, etc.) and complications (e.g. liver dysfunction and rhino-orbital cerebral mucormycosis) leading to high morbidity and even mortality [14–16]. Therefore, regular monitoring of these viruses is essential to detect any mutations and adjust treatment accordingly. The strength of the study lies in the regular assessment of the effects of HGV on liver function and its effectiveness using LFTs as an indicator.

Our study found that surveillance for HGV infection based on liver enzyme abnormalities is not likely to be a major cause of liver disease in high-risk populations and patients with chronic liver diseases. Therefore, HGV is usually ruled out as a cause of liver problems. Liver damage due to HGV cannot be confirmed using an ELISA test with RT-PCR; instead, the virus must be isolated from liver biopsy.

Previous studies have found HGV anti-E2 antibodies in various populations, including healthy blood donors, people with hemophilia and thalassemia, as well as those with chronic liver disease and workers in the field. This suggests the need to include these groups in strategies for controlling and preventing viral hepatitis [17].

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In thalassemia patients, the frequency of HGV RNA was low, and there were no significant differences between patients with normal and elevated ALT levels, consistent with a previous study [2]. Likewise, Ramia et al. (2002) detected a lower prevalence (14.3%) of HGV in Lebanese patients with thalassemia [18].

Co-infection with HGV does not seem to affect the progression of liver disease in chronic hepatitis B or C, based on the monitoring of liver function enzyme levels. This is consistent with previous findings [19]. HGV co-infection may not stimulate the progression of chronic hepatitis B or C as previously thought, based on the current result [6, 20].

The current study was comparable to a previous study that is important in providing insight into the clinical importance of HGV in a region suffering from high rates of viral hepatitis, cirrhosis, liver cancer, and chronic liver diseases, some of which are of unknown cause [21]. High-risk groups such as volunteer blood donors, healthcare workers, hemophilia and thalassemia patients, and blood recipients and donors are most at risk of contracting HGV through acupuncture and multiple blood transfusions [22].

The study suggests that there is a spread of HGV RNA in the blood of patients in risk groups and those with chronic liver disease in Iraq. More research with a larger sample size is needed to confirm this spread, and periodic blood testing for HGV infection in blood donors is considered necessary to preserve blood safety. Anti-HGV-E2 antibodies may offer protection against HGV infection, as suggested by a previous study, this provides a suitable environment for future research that helps in developing preventive methods using anti-E2 antibodies in HGV [21].

The rate of HGV infections in people with occult viral hepatitis is similar to the rate of these infections in people with chronic hepatitis caused by the hepatitis C virus. This suggests that HGV may not be directly harmful to liver disease [1]. In Egypt, 39% of blood donors positive for hepatitis C virus had HGV RNA detected. However, there was no statistical significance between the presence of HGV RNA and hepatitis B virus infection [23]. Some donors positive for HGV RNA show a slight increase in ALT levels, but 75% have normal liver enzymes and no liver symptoms [1, 3].

People with HGV often have normal ALT levels and rarely show liver pathology [1, 2]. Liver function enzyme levels alone may not be sufficient to confirm HGV infection as the cause of viral hepatitis, and liver biopsies are necessary for confirmation [1].

The current study's results were consistent with previous research suggesting that the incidence of HGV infections varies by group of population [2]. In people with a high risk of getting HGV, like those with haemophilia, thalassemia, or uremic, the rate of infection ranges from 14.1% to 38.6% for HGV RNA and from 10.6% to 27.3% for anti-E2 antibodies [24]. Conversely, the prevalence of HGV infection is lower in blood donors, only 3.4% tested positive for HGV RNA and 7.2% tested positive for anti-E2 antibodies [25].

HGV is commonly found in the bodies and tissues of infected individuals but is rarely observed in liver tissue [26]. Despite the presence of the virus, about 75% of infected people have normal liver enzyme levels and show no signs of liver damage [1]. Some HGV-positive blood donors may show liver damage with a slight increase in blood ALT levels, as indicated by several studies [1, 26]. Studies have not found conclusive evidence that HGV causes acute or chronic liver disease, despite its widespread prevalence [1].

Liver enzymes like ALT, AST, ALB, ALP, and total bilirubin are often used to find liver diseases caused by viruses [3]. However, a rise in these enzymes in cases of HGV infection may not always mean liver damage or chronic hepatitis [7].

A small percentage of people with chronic liver disease were found to be infected with HGV, but the virus did not affect the severity of the liver infection [3]. HGV is not significantly involved in cryptogenic liver disease, as the presence of HGV RNA in the serum of patients with this condition is rare. Overall, the evidence does not support a strong association between HGV infection and cryptogenic chronic liver disease [27].

Most HGV infections cause no symptoms, but some people may experience mild to moderate illness with symptoms similar to those of acute viral hepatitis [28]. This is in contrast to the results of the current study. Although chronic HGV infection and viremia have been described, there is rarely evidence of liver injury on biopsy and serum ALT levels are typically normal [26]. Some HGV-positive donors may experience a slight increase in blood ALT levels, but a significant percentage may show no signs of liver damage [5, 26].

The differences in the rates of HGV infections between high-risk groups and patients with chronic liver disease in this study may be due to differences in the study populations within each group as well as differences in the geographical locations in which the study was conducted (Blood centers compared to teaching hospitals in Baghdad and Anbar Provinces).

HGV is a single-stranded RNA virus, meaning it can mutate or recombine quickly. This can cause the infection to cause more damage to the liver. Therefore, regular clinical monitoring and assessment of liver function are necessary to prevent unforeseen consequences. A similar situation occurred with the coronavirus, another RNA virus that has mutated in recent years and repeatedly led to pandemic COVID-19 outbreaks. COVID-19 has resulted in significant morbidity and mortality rates in humans and may also cause gastrointestinal and hepatic manifestations [10].

In addition to viral hepatitis, there are a variety of other causes of abnormal LFTs, including autoimmune diseases, metabolic disorders, toxic liver damage, and non-alcoholic fatty liver disease (NAFLD). In some cases, a liver biopsy may be necessary to assess the severity of liver damage and determine the underlying cause [10, 29, 30].

The article talks about how important it is to check for both HGV anti-E2 antibodies and HGV RNA when figuring out if a patient's LFTs are normal or not. The detection of these markers of active HGV infection may be helpful in the diagnosis and treatment of chronic liver disease. In addition, screening blood donors, healthcare workers, haemophiliacs, and thalassemia patients for signs of exposure to the serious virus and infection can prevent transmission and identify those who need treatment [8]. Comprehensive HGV screening using anti-E2 and RNA detection may therefore have important clinical and public implications.

There were many limitations to the present study. First this investigation relied on ELISA tests and HGV RNA, which may not be enough to check the effects of the virus on the liver. To confirm liver damage, further diagnostic procedures are required, such as the isolation of the virus from liver cells and the histopathology of liver tissue using liver biopsies. The study had a narrow scope, targeting solely high-risk and chronic liver disease patients in Iraq; this was considered a second shortcoming. Therefore, the results cannot be generalizable to other populations or settings. The third limitation

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was that the study only examined the prevalence of HGV infection in these participants and did not assess other relevant risk factors or co-infections. Fourth, the study did not provide information on the specific HGV genotypes identified or other details about the infections. Lastly, the study did not include a control group of healthy people for comparison.

CONCLUSION

Our study found that surveillance for HGV infection based on LFTs abnormalities is not likely to be a major cause of liver disease in high-risk populations and patients with chronic liver disease. Therefore, HGV is usually ruled out as a cause of liver problems. ELISA tests for the detection of HGV RNA may not be sufficient to confirm liver damage. For this reason, further diagnostic procedures, such as virus isolation from liver cells and liver biopsy are required.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

Written approval was obtained from the Ethical Approval Committee of the University of Anbar, Iraq. Study data/information was used for research purposes only. Informed consent was obtained from each participant.

Consent for Publication

No personal data are included.

Availability of Data and Material

The datasets produced and/or analysed during the present study can be obtained from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

All the listed authors have made significant, direct, and intellectual contributions to the work and have approved it for publication.

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