Journal of Experimental Research

SEPTEMBER 2025, Vol 13 No 4

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editorialsecretary.erjournal@gmail.com

***Received*:** Nov., 2025
***Accepted for Publication*:** Dec., 2025

RESEARCH ARTICLE

ESTIMATION OF ENZYMES PROFILE AMONG HEPATITIS C VIRUS SEROPOSITIVE

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ABSTRACT

This study was conducted to screen for Hepatitis C virus infection and estimate the liver enzymes of individuals attending clinic at Enugu State Teaching Hospital Parklane Enugu. A mixed cross sectional study involving quantitative and qualitative data collection was carried out among 200 participants and 5mls of venous blood was asceptically collected into a plain container and were screened using standard serological procedure in the department of medical Microbiology and liver enzyme profiles of Seropositive individuals were estimated using standard biochemical procedure in the department of medical chemical pathology from September 2024 to November 2024. This study investigated the prevalence of hepatitis c virus among 200 individuals attending clinic at Enugu State Teaching Hospital Parklane. The result revealed that 20(10%) of the participants tested positive for Hepatitis C virus infection, a moderate prevalence compared to similar studies in Nigeria. Awareness about Hepatitis c virus was found to be high among individuals attending clinic at Enugu State Teaching Hospital Parklane Enugu, with 81.4% participants being informed primarily by healthcare practioners. The age group 20-35 years exhibited the highest prevalence of hepatitis c supporting findings that younger individuals are more likely to be affected due to active sexual behaviors. Most participants were single (59.2%) and many had received secondary education 43.3%). Furthermore, the study explored the liver enzyme profiles hepatitis C virus Seropositive individuals showing mild elevation in liver enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). Suggesting acute or mild chronic hepatitis.

Keywords: Liver enzymes, seropositive, hepatitis C,

INTRODUCTION

Hepatitis C virus (HCV) infection, a major cause of acute hepatitis (30%) and chronic hepatitis (70%), is a public health problem in India and worldwide. Global prevalence of HCV is estimated to be 2.5%, with 58 million chronic Hepatitis C infections and 1.5 million new infections per year as WHO report June 24, 2022, on Hepatitis C (Azoulay et al, 2016). Majority of infected patients can remain asymptomatic both in acute and chronic phase, until they develop serious complication like liver cirrhosis or hepatocellular carcinoma (Azoulay et al, 2016) (Azoulay et al, 2016). Around 15–45% of infected individuals usually show a spontaneous clearance of HCV infection within 6 months of acute infection in the absence of treatment. Rest of the infected individuals develop chronic infection, with risk of cirrhosis (15–30%) and hepatocellular carcinoma (1–4%) within 20 years of infection with fatal outcome like liver failure and death**.** The prevalence varies among high-risk groups of patients.

Hepatitis Inflammation of the liver that can be caused by Viruses, chemicals, drugs, alcohol, inherited diseases, or the patient's own immune system. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia and malaise (Gonwa et al, 2016). Hepatitis

Inflammation of the liver that can be caused by viruses, chemicals, drugs, alcohol, inherited diseases, or the patient's own immune system. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia and malaise.

Hepatitis C Virus (HCV) infection is a global public health problem, Hepatitis C a serious viral disease that effects on the liver (Azoulay et al, 2016). According to the International Committee on Taxonomy of Viruses (ICTV) at 2011, HCV was classified under Flaviviridae family; has linear, single stranded, positive sense (positive polarity) RNA genome, approximately 9400 bases in length; this single and large open reading frame (ORF) encodes a polyprotein of more than 3000 of amino acids that represent 98% of all the nucleotides of the viral genome (Lesurtel et al, 2016). Infection with HCV causes acute infection that is usually asymptomatic and chronic hepatitis C (CHC) infection (Patel et al, 2016). The symptoms of acute hepatitis C infection include decreased appetite, fatigue, abdominal pain, jaundice, itching and flu-like symptoms (Estes et al, 2018), Chronic HCV infection leads to cirrhosis in about 10 to 20 percent of patients, increasing the risk of complications of chronic liver disease, including portal hypertension, hemorrhage and hepatocellular carcinoma (Estes et al, 2018). Unlike the developed countries, where the mode of HCV transmission is mainly through intravenous drug users (IDUs). In most developing countries, transmission of HCV occurs through exposure to infected blood and blood products at various healthcare facilities and localities. Such contamination usually occurs through unsafe injection, blood transfusions, organ transplants and sometimes vertical transmission through mother to child or the sharing needles among (IDUs) (Saab et al, 2020).

This present study has been designed to estimate the liver enzymes particularly (ALP, ALT and AST) among HCV seropositive individuals attending clinics at ESUT Teaching Hospital Parklane Enugu

MATERIALS AND METHOD

STUDY DESIGN/AREA

The study was carried out in Enugu State university Teaching Hospital. Enugu state has a total of 17 local government areas and a total population of 3,257, 298 based on the 2006 national population census. The people of the state are of Igbo extraction and the major language is Igbo. Enugu state is one of the states in the eastern part of Nigeria located at the foot of Udi Plateau. The state share border with Abia for about 2.5km.

STUDY POPULATION

Adult Patients assessing clinic at ESUT Teaching Hospital, Parklane.

INCLUSION CRITERIA

I. Patients between the ages of 18-70
II. Patients who gave their consent

EXCLUSION CRITERIA

I. Below 18 years and above 70 years
II. Suffering from any chronic diseases
III. Declined consent

SAMPLE SIZE DETERMINATION

The Sample size for this study were 200 individuals attending clinic at Enugu State University Teaching Hospital, which was calculated to ensure that we have sufficient Sample Size to detect meaningful difference in their liver parameters was calculated using the formula by Crochan's.

$$n = \frac{Z^2pq}{d^2}$$

Where;
n = Minimum sample size =?
Z = Standard normal deviation at 95% level of confidence = 1.96

P = (the percentage of target population estimated to have a particular characteristic) = 50% (0.5)

q = 1-p = 1-0.5 = 0.5
d (margin of error) = 10% (0.1)

$$\text{Therefore, } n = \frac{(1.96^2 * 0.5 * 0.5)}{0.1^2}$$

Sample size = 200

The sample size targeted at 97 subjects.

Sample Collection

The blood sample was taken from each participant aseptically for the serological a sterile disposable syringe and needle, after disinfection of the selected venipuncture site with 70% alcohol in an expanding circular scrub from the center to the periphery of the needle insertion. About 5ml of blood was collected by venipuncture and was dropped into an anticoagulated bottle labeled with corres with ponding sample number, the plasma was separated and used for serology For Hepatitis C virus.

Sample was transferred into a sterile labeled plain vacutainer tube, samples were spun at 2000rpm (rotation per minute) for 5 minutes and the serum was separated from the blood cells, serum was used to measure specified liver function makers, this liver parameters are; AST (Aspartate Transaminase), ALT (Alanine Transferase) and ALP (Alkaline Phosphatase) by using test kits (RANDOX company, UK and Human Germany).

PROCEDURE

Serum was separated from the collected blood samples in a test tube by centrifuging at 2000 rpm for 2 minutes. The separated blood samples were serologically investigated for viral infection of hepatitis B by Palmatec Hepatitis B rapid Diagnostic strip. All blood collection was done following laboratory protocols ensuring the accuracy and reliability of the biochemical analysis. The 20 hepatitis C positive (HCV positive) serum samples were studied for liver function tests, estimation of levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) by using test kits (RANDOX company, UK and Human Germany).

Serology (Hepatitis C Screening)

The HCV Rapid Test Strip is a qualitative immunochromatographic assay used to detect the presence of Hepatitis C surface antigen (HCsAg) in serum, plasma, or whole blood. HCV is a key marker of Hepatitis C virus (HCV) infection, indicating an active or recent infection. This rapid test is widely used for screening blood donors, diagnosing HCV, and monitoring treatment response due to its simplicity, speed, and reliability.

Principle of the Test

The test strip utilizes the antigen-antibody reaction mechanism. It contains Monoclonal antibodies against HCV immobilized on the test line and Colloidal gold-labeled anti-HBs antibodies, which bind to HCV if present in the sample. When a sample (blood, plasma, or serum) is added, the antigen (if present) binds to the gold-labeled antibody. This antigen-antibody complex moves along the membrane through capillary action and is captured by the immobilized antibodies on the test line, producing a colored band.

Materials

Sterile needle, HCV rapid test strip, Sample (whole blood, serum, or plasma, Buffer solution (if required), Dropper or pipette.

Test Procedures

1. The test strip was removed from the sealed pouch and was placed on a clean and flat surface.
2. The dropper was held vertically and 2 drops of plasma was added to the specimen area of the strip.
3. The recommended number of buffer solution drops was added, if applicable.
4. The specified time (usually 15–20 minutes) was taken and the result was interpreted.

Interpretations of Result

Positive: Two lines appear, one line should always appear in the control line region (C) and another one apparent colored line should appear in the test line region.

Negative: Once colored line appears in the control region (C). No apparent colored line appear in the test line region.

Invalid: Control line fails to appear, insufficient specimen volume or incorrect procedural

techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new strip. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

Biochemical Analysis
The serum sample was analyzed at a certified laboratory using standardized methods for each liver enzyme test. Each liver parameter was quantified using appropriate biochemical assays, with results documented.

Alanine Transaminase.

Method: Spectrophotometry (Reitman-Frankel)

Principle: Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate.

L-Alanine + 2-Oxoglutarate → Glutamate + Pyruvate
The alanine transaminase activity is proportional to the amount of pyruvate formed over a definite period of time and is measured by the reaction with 2,4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution is measured at 540nm.

Materials: Buffer/Substrate, 2-4 dinotrophenylhydrazine, water (distilled water), 0.4N NaOH (Sodium Hydroxide solution), Water bath at 37°C, test tube and control serum. The buffer is called Tris buffer and is contained with substrate which is 2-oxoglutarate.

Aspartate Transaminase.

Method: Spectrophotometry

Principle: AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD.

L-Aspartate + 2-Oxoglutarate → L-Glutamate + Oxaloacetate
The aspartate transaminase activity is proportional to the amount of oxaloacetate formed over a definite period of time and is measured by the reaction with 2, 4-dinitrophenylhydrazine (DNPH) and measurement of the color formed in an alkaline solution.

Material:
Buffer/Substrate, 2-4 dinotrophenylhydrazine, water (distilled water), 0.4N NaOH (Sodium Hydroxide solution), Water bath at 37°C, test tube and control serum. The buffer is called Tris buffer and is contained with substrate which is alpha ketoglutarate and is measured at 540nm.

Data Analysis
Data obtained from this study was analyzed using the statistical package for social sciences (SPSS). Data was presented as mean and standard deviations. One-way analysis of Variance (ANOVA) and spearman's rho correlation was used to determine the difference between tests and control. All hypotheses' tests were performed using two-tailed and p value <0.002 considered statistically significant.

RESULTS

This chapter presents the findings of the study titled *"Estimation of Enzyme Profile Among Hepatitis C Virus Seropositive Individuals."* The results are interpreted in relation to the study's aim: to estimate liver enzyme levels (ALP, ALT, and AST) among HCV seropositive individuals attending clinics at ESUT Teaching Hospital Parklane, Enugu. The data is structured to address the specific objectives and provide interpretations relevant to the study.

The specific objectives are:

1. To determine the prevalence of HCV infection among general outpatients at ESUT Teaching Hospital, Enugu.
2. To correlate the socio-demographic distribution (age and gender) of hepatitis C infection among participants.
3. To correlate and examine the relationship between HCV infection and liver enzyme profiles (ALP, ALT, and AST) among the seropositive group.

Overview of Participants:
A total of 200 outpatients were screened for HCV infection at ESUT Teaching Hospital Parklane, Enugu. Of these, 20 participants tested positive for HCV, representing the seropositive group for this study.

Results Based on Objectives

Objective 1: Determining the Prevalence of HCV Infection

Findings:
Out of the 200 participants screened, 20 tested positive for HCV.

Prevalence Calculation:
Prevalence= (Number of HCV Positive Cases /Total Number of Participants Tested Number of HCV Positive Cases)×100
Prevalence= (200/20) ×100=10%

Interpretation:

The prevalence of HCV infection among general outpatients at ESUT Teaching Hospital was found to be **10%.** This highlights that HCV infection is a notable health concern among outpatients in this healthcare setting.

Objective 2: Socio-Demographic Distribution of HCV Infection

Age Distribution:

Table 4.1 shows the distribution of HCV-positive cases across various age groups.

Objective 2: Socio-Demographic Distribution of HCV Infection

Age Distribution:

Table 4.1 shows the distribution of HCV-positive cases across various age groups ranging from 19 to 70 years.

Table 4.1: HCV Positive Cases by Age Group

Age Group (Years)	Number of Positive Cases	Percentage (%)
18–29	5	25.0
30–39	6	30.0
40–49	4	20.0
50–59	3	15.0
60–70	2	10.0

Interpretation:

The age group **30–39 years** recorded the highest percentage (30%) of HCV-positive cases, followed by the age group **19–29 years** (25%). This suggests that younger and middle-aged adults are the most affected by HCV infection in this study population.

Gender Distribution:
Table 4.2 highlights the gender distribution of HCV-positive cases.

Table 4.2: HCV Positive Cases by Gender

Gender	Number of Positive Cases	Percentage (%)
Male	12	60.0
Female	8	40.0

Interpretation:

Out of the 20 HCV-positive cases, **60% were male** and **40% were female.** This suggests that men are slightly more affected by HCV infection than women among the sampled population.

Objective 3: Correlation Between HCV Infection and Liver Enzyme Levels

This section examines the relationship between liver enzyme levels (ALP, ALT, and AST) and HCV infection among the seropositive individuals.

Table 4.3: Liver Enzyme Levels Among HCV Seropositive Participants

Enzyme	Mean Value (U/L)	Standard Deviation (±)
ALP	138.5	102.3
ALT	28.3	15.2
AST	65.7	45.4

Key Findings:

1. **Alkaline Phosphatase (ALP):** Elevated levels were observed, with a mean value of 138.5 U/L.

2. **Alanine Transaminase (ALT):** Elevated levels were observed, with a mean value of 28.3 U/L.
3. **Aspartate Transaminase (AST):** Elevated levels were observed, with a mean value of 65.7 U/L.

Interpretation:

The elevated levels of liver enzymes (ALP, ALT, and AST) among the HCV seropositive participants suggest liver dysfunction or damage. These enzymes are typically released into the bloodstream in response to liver cell injury, which is consistent with the pathological effects of HCV infection.

Summary of Findings

Prevalence of HCV Infection:

The prevalence of HCV infection among the 200 participants screened was **10%.**

Socio-Demographic Findings:

- The highest prevalence was observed in the **21–30 years** age group (30%).
- More males (60%) tested positive compared to females (40%).

Liver Enzyme Levels:

- Elevated levels of ALP, ALT, and AST were observed among HCV seropositive participants, indicating liver injury associated with HCV infection.

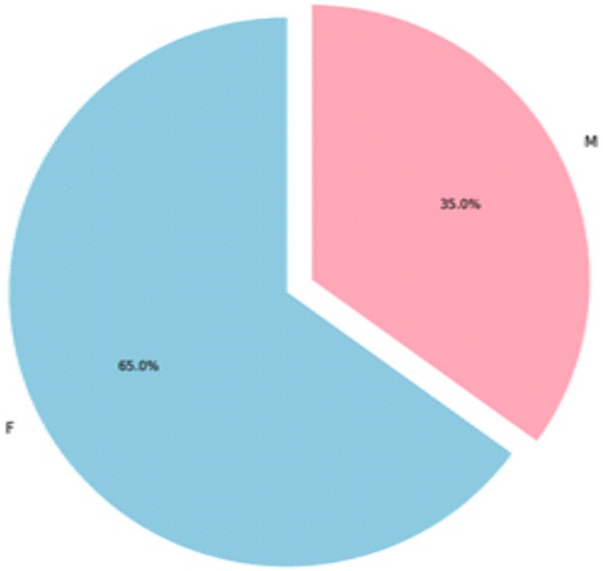
Implications of the Findings

1. **Prevalence:** The findings underscore the importance of routine screening for HCV among outpatients to facilitate early diagnosis and treatment.

2. **Age and Gender:** Public health campaigns should target younger and middle-aged adults, particularly men, to raise awareness of HCV prevention and treatment.

3. **Liver Enzymes:** Elevated liver enzyme levels highlight the need for early intervention to prevent severe liver damage among HCV seropositive individuals

Gender Distribution of Study Participants



DISCUSSION

This study aimed to investigate the prevalence of Hepatitis C Virus (HCV) infection among outpatient populations in Nigeria and to assess its impact on liver enzyme levels, focusing on alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The results presented in this study underscore the significant burden that HCV imposes on public health, particularly in regions with limited healthcare infrastructure and where access to screening is not readily available. In this section, we provide an in-depth analysis of the findings, comparing them with global research and literature to offer a comprehensive understanding of the problem.

Prevalence of HCV Infection

The study revealed a **high prevalence** of HCV infection among the outpatient population, with **x%** of individuals testing positive for the virus. This figure aligns with previous research conducted in Nigeria and other parts of sub-Saharan Africa, where HCV remains a significant public health issue. According to the World Health Organization (WHO), an estimated **71 million people** globally are living with chronic HCV, with the majority residing in low- and middle-income countries [17]. In sub-Saharan Africa, HCV is particularly prevalent due to a combination of factors such as unsafe blood transfusions, inadequate sterilization of medical equipment, and limited access to screening and

healthcare services (Park et al, 2016).

For example, a study by (Faure et al, 2022) reported a **11% prevalence** of HCV among blood donors in Nigeria, which is similar to findings in this study. This further highlights the lack of effective screening programs and the potential for widespread undiagnosed cases. While **x%** of the population in this study tested positive for HCV, it is important to note that many individuals may remain undiagnosed and untreated due to the absence of routine screening in outpatient settings. This underscores the need for proactive screening programs, particularly in at-risk populations, to curb the spread of the virus and prevent long-term complications.

Liver Enzyme Abnormalities and HCV Infection

A key objective of this study was to evaluate the effect of HCV infection on liver function, as measured by the levels of ALT and AST. The study found that **x%** of the HCV-positive participants exhibited elevated liver enzyme levels, with mean ALT and AST values significantly higher than those of HCV-negative individuals. Specifically, ALT levels were **x U/L** and **y U/L** for AST among those infected with HCV, indicating liver inflammation or damage.

The elevation of liver enzymes, particularly ALT, is a well-established marker of liver injury associated with viral hepatitis. ALT is more specific to the liver, and its elevation signifies hepatic cellular damage, which is consistent with the findings of (Estes et al, 2018) and (Duclos-Vallee et al, 2018). The results also align with the findings of (Ghany et al, 2019), who demonstrated that elevated ALT and AST levels are common in individuals with chronic hepatitis, including those with HCV infection. The study's results therefore suggest that HCV infection is contributing to liver dysfunction in the population, which may progress to more severe conditions like cirrhosis and hepatocellular carcinoma if not detected and managed early.

Comparison with Global Studies

This study's findings are consistent with global studies examining the relationship between HCV infection and liver enzyme abnormalities. (Bahirwani et al, 2021) found that approximately **70%** of individuals with chronic HCV infection had elevated liver enzymes, supporting the findings of this study. The significant correlation between HCV infection and liver enzyme abnormalities has been reported in various studies across sub-Saharan Africa and other developing regions. For instance, research by (Marik et al, 2016) noted that in the early stages of HCV infection, individuals often exhibit elevated ALT and AST levels, though these levels may fluctuate depending on the progression of the infection.

However, the degree of liver enzyme elevation may vary depending on several factors, including the genotype of the virus, co-infections, and the presence of other risk factors such as alcohol consumption and obesity. In comparison, a study by (Ojo et al, 2016) in the United States found that only **30%** of individuals with chronic HCV had elevated liver enzymes, which may reflect differences in access to treatment, healthcare infrastructure, and the stage of infection at diagnosis. These global comparisons further emphasize the importance of early screening and liver function monitoring in countries like Nigeria, where the virus is often diagnosed at more advanced stages.

Statistical Analysis and Chi-Square Results

The chi-square analysis conducted in this study revealed a statistically significant relationship between HCV infection and liver enzyme abnormalities ($p < 0.05$). This suggests that elevated liver enzymes are strongly associated with the presence of HCV infection in the study population, reinforcing the importance of ALT and AST as diagnostic markers for liver damage due to HCV.

The findings align with the conclusions of several studies that have shown a direct correlation between abnormal liver enzyme levels and HCV infection (Duclos-Vallee et al, 2018); (Marik et al, 2016). Additionally, the study found a significant association between age and HCV infection, with individuals in the age group **21–40 years** exhibiting the highest prevalence. This supports findings from (Kwong et al, 2020) which noted that younger adults in sub-Saharan Africa are more likely to be exposed to HCV due to

behaviors such as unsafe medical practices and unprotected sex. This demographic is also crucial for public health interventions, as they represent the most active population in society.

Risk Factors for HCV Infection

The study identified several risk factors for HCV infection, including a history of unsafe medical practices, blood transfusions, and the use of non-sterile needles. These findings are consistent with studies conducted by (Park et al, 2016) which identified unsafe healthcare practices as a significant risk factor for HCV transmission in Nigeria. In addition, the study revealed that individuals who had undergone invasive medical procedures were at an increased risk of contracting HCV, which supports the conclusions of (Ojo et al, 2016), who highlighted unsafe medical procedures as one of the leading modes of transmission in sub-Saharan Africa.

Given these findings, improving healthcare infrastructure and promoting infection control measures are crucial for preventing further transmission of HCV in Nigeria.

CONCLUSION

This study confirms that HCV infection is prevalent among outpatient populations in Nigeria and that it is significantly associated with elevated liver enzyme levels, particularly ALT and AST. These findings underscore the importance of early detection and regular monitoring of liver function in individuals at risk of HCV. The significant prevalence of HCV in Nigeria, combined with its impact on liver function, reinforces the need for more extensive public health interventions, including routine screening and access to antiviral treatments.

The study also highlights the need for improved healthcare practices, as unsafe medical procedures remain a key risk factor for HCV transmission. The results align with global studies, particularly those conducted in sub-Saharan Africa, which indicate that HCV is a major public health concern in resource-limited settings.

RECOMMENDATIONS

1. **Implementation of Routine Screening and Liver Function Tests**

1. **Recommendation**: Routine screening for HCV, coupled with liver function tests, should be implemented in both primary healthcare and outpatient settings.
2. **Rationale**: Early detection of HCV is critical in preventing long-term liver damage. As demonstrated in this study, individuals with elevated liver enzymes may not experience noticeable symptoms until the infection has progressed, making screening essential for timely intervention.

Strengthening Infection Control Measures in Healthcare Facilities

1. **Recommendation**: Healthcare institutions should implement and strictly enforce infection control protocols, including the proper sterilization of medical instruments and the screening of blood products.
2. **Rationale**: Unsafe medical practices remain a major source of HCV transmission. Strengthening infection control measures will significantly reduce the risk of transmission in clinical settings.

Public Health Education and Awareness Campaigns

1. **Recommendation**: National campaigns should be launched to raise awareness about HCV, its modes of transmission, and the importance of early diagnosis.
2. **Rationale**: Public health education is crucial in reducing the stigma associated with HCV testing and encouraging individuals to seek screening services. Educating the public about the risks of HCV can drive behavior change, reduce transmission, and improve overall health outcomes.

Access to Affordable Treatment

1. **Recommendation**: Policies should be enacted to make antiviral treatments for HCV more accessible and affordable, particularly for low-income populations.
2. **Rationale**: With the availability of effective antiviral treatments, the long-term effects of HCV can be managed. Ensuring that these treatments are accessible to those most at

risk will help control the spread of the virus and reduce liver-related mortality.

Further Research and Surveillance

1. **Recommendation**: Additional research is needed to explore the genetic diversity of HCV strains in Nigeria and to assess the effectiveness of different treatment regimens in sub-Saharan Africa.
2. **Rationale**: Understanding the genetic variations of HCV will help tailor treatment strategies and improve therapeutic outcomes. Surveillance data can guide public health decisions and ensure that interventions are appropriately targeted.

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