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Prevalence and genotyping of hepatitis B and C viruses in patients attending hospitals in Libya

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Abstract: Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections continue to represent a substantial public health burden due to their long-term clinical consequences, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. This study aimed to assess the prevalence and molecular genotype distribution of HBV and HCV among patients attending hospitals and diagnostic laboratories in Sabratha City, Western Libya. The study was conducted from June to December 2025. A total of 420 serum samples were obtained from adult patients undergoing routine screening for HBV and/or HCV infection. Serological testing was performed using an enzyme-linked immunosorbent assay to detect hepatitis B surface antigen (HBsAg) and anti-HCV antibodies. Molecular detection and genotyping were carried out using polymerase chain reaction-based methods in seropositive samples. Overall, 6.9% of the study population tested positive for viral hepatitis. HBV infection was detected in 4.0% of the participants, while HCV seropositivity was observed in 2.9%. Both infections were more frequently identified among male patients, although no significant gender differences were observed. HBV DNA was successfully genotyped in the majority of HBsAg-positive samples, with genotype D identified as the predominant genotype, followed by genotype A and mixed genotypes. Among HCV RNA-positive samples, genotype 4 was the most prevalent, followed by genotype 1, with a small proportion of mixed infections. These findings demonstrate that HBV and HCV infections remain prevalent among patients attending healthcare facilities in Western Libya, with HBV showing a higher burden than HCV. The predominance of HBV genotype D and HCV genotype 4 is consistent with regional molecular epidemiological patterns and has important implications for clinical management, treatment selection, and public health planning. Continued molecular surveillance and expanded screening strategies are essential to support national hepatitis control and elimination efforts.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections remain major global public health concerns, contributing significantly to chronic liver disease, cirrhosis, and hepatocellular carcinoma despite the existence of effective vaccines and antiviral therapies. The virus is commonly transmitted from mother to child during birth and delivery, and through contact with blood. According to the World Health Organization (WHO), an estimated 304 million people worldwide were living with chronic hepatitis B or C infection in 2022, and viral hepatitis was responsible for approximately 1.3 million deaths annually, largely due to liver-related complications such as cirrhosis and liver cancer [1, 2]. Although safe and effective vaccines are available for hepatitis B and curative treatments exist for hepatitis C, a large proportion of infected individuals remain undiagnosed or untreated, particularly in low- and middle-income regions [1, 2].

The epidemiology of HBV and HCV infection exhibits pronounced geographical variation, influenced by differences in vaccination coverage, healthcare infrastructure, blood safety practices, and population-level behavioral and socioeconomic factors [1, 3]. In addition to prevalence estimates, understanding the genetic diversity of these viruses is of considerable clinical importance. Distinct HBV genotypes have been shown to differ in their associations with disease progression, response to antiviral therapy, and risk of complications such as liver fibrosis and hepatocellular carcinoma [4]. Likewise, HCV genotypes influence treatment selection and sustained virological response rates, making genotype determination an integral component of clinical management and public health planning. In many countries where comprehensive population-level surveillance is limited, hospital-based and clinical cohort studies provide critical epidemiological data. These studies often capture a broad spectrum of patients, ranging from symptomatic cases to asymptomatic individuals identified through routine screening, thereby offering valuable insights into circulating viral strains, prevalence patterns, and clinical characteristics within specific regions. In Libya, national seroepidemiological surveys have estimated the prevalence of chronic HBV and HCV infections in the general population, with HBsAg and anti-HCV seropositivity reported at approximately 2.2% and 1.2-1.3%, respectively [5-7]. However, detailed data on viral genotype distribution among patients attending healthcare facilities, particularly in subnational regions such as Western Libya, remain limited. Existing molecular studies in Libya have demonstrated that HCV genotypes 1 and 4 are among the most prevalent in clinical settings, with notable regional variation in genotype frequencies [8]. Evidence from these studies underscores the need for updated, region-specific genotype data to inform targeted diagnostic, therapeutic, and preventive strategies. Thus, the current study aims to evaluate the prevalence and genotype distribution of HBV and HCV among patients attending hospitals in Libya. By generating updated epidemiological and molecular data, this work seeks to enhance understanding of the current burden and diversity of viral hepatitis in the region and to support evidence-based public health and clinical interventions.

Materials and methods

Study design: This was a hospital-based cross-sectional study located in Western Libya. The study was carried out in collaboration with major public hospitals and diagnostic laboratories in Sabratha, including hospital outpatient clinics and central medical laboratories that routinely provide serological and molecular diagnostic services. The study period extended from 30 June 2025 to 30 December 2025.

Study population: During the study period, a total of 420 blood samples were collected from patients attending hospitals and medical laboratories in Sabratha for hepatitis B and or hepatitis C testing. The sample size reflects all eligible individuals who met the inclusion criteria during the defined timeframe.

Inclusion criteria: Patients aged more than 18 years, individuals who provided blood samples for hepatitis B and/or hepatitis C testing, and patients who gave informed consent to participate in the study.

Exclusion criteria: Patients with incomplete clinical or laboratory data, individuals with a documented history of antiviral treatment for HBV or HCV prior to sample collection, and repeat samples from the same patient during the study period.

Sample collection: 5.0-10.0 mL venous blood samples were collected from each participant under aseptic conditions. Samples were centrifuged to separate serum, which was aliquoted and stored at -20°C until analysis. All the samples were handled and processed in accordance with the standard biosafety and laboratory quality control procedures in the participating laboratories.

Serological testing: The screening was performed by commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' advices. HBV infection was determined by the detection of hepatitis B surface antigen (HBsAg). HCV infection was determined by the detection of anti-HCV antibodies. Positive and negative controls were included in each assay run to ensure test validity and reliability.

Molecular detection and genotyping: Samples that tested positive for HBsAg and or anti-HCV antibodies were subjected to molecular analysis. Viral nucleic acids were extracted from the serum samples using standardized commercial extraction kits. HBV DNA and HCV RNA were detected using polymerase chain reaction (PCR) or real-time PCR (RT-PCR) techniques. Genotyping of HBV and HCV was performed using genotype-specific primers and probes, or commercially available genotyping assays, following established protocols. The genotype results were classified according to internationally accepted HBV and HCV genotype nomenclature.

Data collection: Demographic and clinical data, including age, gender, and reason for testing, were collected using structured data collection forms. All data were anonymized to maintain patient confidentiality.

Ethical considerations: The study protocol was reviewed and approved by the local ethics committee and hospital administration in Sabratha (Sab-L: 03-2025). Written informed consent was obtained from all participants prior to sample collection. All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical analysis: Prevalence of HBV and HCV infections was calculated as a percentage. Genotype distributions were expressed as frequencies and proportions. Descriptive statistics were used to summarize demographic and laboratory findings. A $p<0.05$ was considered statistically significant where applicable.

Results

In **Table 1**, during the study period, a total of 420 patients attending hospitals and medical laboratories in Sabratha City, Libya, were enrolled. The participants included 238 males (56.7%) and 182 females (43.3%), with a mean age of 41.8 ± 13.6 years (range: 18-79 years). In **Table 2**, 6.9% patients were positive for either HBV or HCV infection. HBsAg positivity was detected in 4.0% patients, and Anti-HCV antibodies were detected in 2.9% patients. In **Table 3**, HBV infection was more frequent among males (64.7%) compared to females (35.3%). Similarly, HCV infection showed a higher prevalence among males (58.3%) than females (41.7%), although the difference was not significant. HBV DNA was successfully detected and genotyped in 88.2% HBsAg-positive samples. Genotype D was the most prevalent (66.7%), genotype A accounted for 20.0% and mixed genotypes (A/D) were detected in 13.3% of cases (**Table 4**). In **Table 5**, HCV RNA was detected in 83.3% anti-HCV positive samples. Genotype analysis revealed that genotype 4 as the predominant genotype (60.0%), Genotype 1 in 30.0% of cases, and mixed genotypes (1/4) in 10.0% of patients. **Figure 1** shows the prevalence of HBV and HCV infections among patients attending hospitals and medical laboratories in Sabratha City during the study period (30 June 2025 to 30 December 2025). HBV infection, determined by HBsAg positivity, was detected in 4.0% of participants, whereas anti-HCV antibodies were identified in 2.9% of the study population.

Table 1: Demographic characteristics of the study population

Characteristics	Frequency	Percentage
Gender		
Male	**238**	**56.7**
Female	**182**	**43.3**
Age group (years)		
18 - 29	**78**	**18.6**
30 - 44	**142**	**33.8**
45 - 59	**121**	**28.8**
≥ 60	**79**	**18.8**

Table 2: Prevalence of hepatitis B virus and hepatitis C virus among Libyan patients

Infection status	Frequency	Prevalence (%)
HBsAg positive	**17**	**4.0**
Anti-HCV positive	**12**	**2.9**
HBV/HCV negative	**391**	**93.1**
Total	**420**	**100**

Table 3: Distribution of hepatitis B virus and hepatitis C virus infections according to the gender

Infection	Male n (%)	Female n (%)	Total
Hepatitis B virus (HBsAg)	**11 (64.7)**	**6 (35.3)**	**17**
Hepatitis C virus (Anti-HCV)	**07 (58.3)**	**5 (41.7)**	**12**

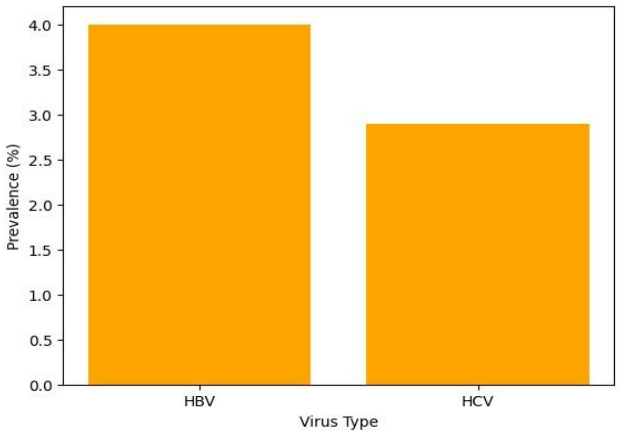
Table 4: Distribution of hepatitis B virus genotypes among hepatitis B virus-positive patients

Hepatitis B virus genotype	Frequency	Percentage
Genotype D	**10**	**66.7**
Genotype A	**03**	**20.0**
Mixed (A/D)	**02**	**13.3**

Table 5: Distribution of HCV genotypes among HCV-positive patients

Hepatitis C virus genotype	Frequency	Percentage (%)
Genotype 4	6	60.0
Genotype 1	3	30.0
Mixed (1/4)	1	10.0

Figure 1: Prevalence of hepatitis B virus and hepatitis C virus among the participants



HBV: Hepatitis B virus and HCV: Hepatitis C virus

Discussion

The present study provides updated data on the prevalence and genotype distribution of HBV and HCV among patients attending hospitals and medical laboratories in Sabratha City, Libya. The findings highlight that viral hepatitis remains a significant public health concern in the region, with HBV infection showing a higher prevalence than HCV infection. The prevalence of HBV infection was higher than that of HCV infection. This

pattern is consistent with previous national and regional studies conducted in Libya, which have consistently reported a higher burden of HBV compared to HCV in the general population and clinical settings [5, 6, 9]. The relatively higher prevalence of HBV may be attributed to perinatal and early childhood transmission, as well as incomplete vaccination coverage in certain age groups, particularly among adults born before the widespread implementation of the national hepatitis B vaccination program [7, 10]. The observed HCV prevalence in the current study is comparable to rates reported in other parts of western Libya and neighboring North African countries, where HCV prevalence generally ranges between 1.0% and 3.0% in the general population [6, 11]. Despite the availability of highly effective direct-acting antiviral therapies, HCV continues to circulate, likely due to undiagnosed infections and ongoing risk factors such as unsafe medical practices and historical blood transfusions prior to systematic screening [12]. Gender-based analysis revealed a higher proportion of HBV and HCV infections among males compared to females. This finding has widely been documented in previous studies and may reflect increased exposure of males to behavioral and occupational risk factors, including invasive medical procedures, blood exposure, and higher mobility [8, 13]. However, the lack of significant gender differences suggests that viral hepatitis remains a risk for both sexes in the studied population.

In this study, genotype analysis demonstrated that HBV genotype D was the predominant genotype detected among HBV-infected Libyan patients. This finding is in agreement with earlier molecular studies from Libya and the Mediterranean region, where genotype D has been shown to be the dominant circulating HBV genotype [4, 7, 14]. HBV genotype D has been associated with more severe liver disease and variable response to interferon-based therapy, underscoring the clinical importance of genotype determination [4, 14]. Regarding HCV, genotype 4 was the most prevalent genotype identified, followed by genotype 1. This distribution mirrors previous reports from Libya and other North African and Middle Eastern countries, where genotype 4 predominates [7, 11, 15]. The predominance of genotype 4 has important therapeutic implications, as treatment response and regimen selection may vary by genotype, despite the broad efficacy of newer antiviral agents [15, 16]. The findings of this study emphasize the continued need for strengthened hepatitis surveillance and molecular epidemiology studies at the subnational level. Hospital-based studies such as this provide valuable insights into circulating viral strains and infection patterns, particularly in settings where population-level surveillance remains limited [17]. Nevertheless, the study has some limitations. The hospital-based design may limit the generalizability of the findings to the wider community, and the relatively short study period may not capture seasonal or long-term trends. Despite these limitations, the study offers important and updated data on HBV and HCV prevalence and genotypes and contributes to the existing body of evidence on viral hepatitis epidemiology in Libya.

Conclusion: The findings demonstrate that both infections remain prevalent in the studied population, with HBV showing a higher prevalence than HCV. The predominance of HBV genotype D and HCV genotype 4 reflects the established molecular epidemiological patterns reported in Libya and the broader Mediterranean and North African regions. These genotype distributions have important clinical and public health implications, particularly in relation to disease progression, treatment response, and the planning of targeted antiviral strategies. The results underscore the value of hospital-based surveillance in generating region-specific epidemiological and molecular data, especially in settings where comprehensive population-level surveillance is limited.

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Author declarations: The author confirms that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.
Generative AI disclosure: No Generative AI was used in the preparation of this manuscript.

انتشار فيروسات التهاب الكبد B و C وتحديد أنماطها الجينية لدى المرضى المراجعين للمستشفيات في ليبيا

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الملخص: لا تزال عدوى فيروس التهاب الكبد ب (HBV) وفيروس التهاب الكبد ج (HCV) تشكل عبئًا كبيرًا على الصحة العامة نظرًا لعواقبها السريرية طويلة الأمد، بما في ذلك التهاب الكبد المزمن، وتليف الكبد، وسرطان الخلايا الكبدية. هدفت هذه الدراسة إلى تقييم مدى انتشار فيروس التهاب الكبد ب وفيروس التهاب الكبد ج وتوزيع الأنماط الجينية الجزيئية بين المرضى المراجعين للمستشفيات ومختبرات التشخيص في مدينة صبراتة، غرب ليبيا. أُجريت الدراسة في الفترة من يونيو إلى ديسمبر 2025. تم الحصول على 420 عينة مصل من مرضى بالغين يخضعون لفحص روتيني للكشف عن عدوى فيروس التهاب الكبد ب و/أو فيروس التهاب الكبد ج. أُجري الاختبار المصلي باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA) للكشف عن مستضد سطح التهاب الكبد ب (HBsAg) والأجسام المضادة لفيروس التهاب الكبد ج. أُجري الكشف الجزيئي وتحديد النمط الجيني باستخدام طرق تعتمد على تفاعل البوليميراز المتسلسل (PCR) في العينات المصلية الإيجابية. بشكل عام، أظهرت نتائج اختبارات 6.9% من المشاركين في الدراسة إصابتهم بالتهاب الكبد الفيروسي. تم الكشف عن عدوى التهاب الكبد B لدى 4.0% من المشاركين، بينما لوحظت إيجابية المصل لالتهاب الكبد C لدى 2.9%. كانت كلتا العدوى أكثر شيوعًا بين المرضى الذكور، على الرغم من عدم وجود فروق جوهرية بين الجنسين. تم تحديد النمط الجيني لحمض HBV النووي بنجاح في غالبية العينات الموجبة لمستضد HBsAg، حيث كان النمط الجيني D هو النمط السائد، يليه النمط الجيني A ثم الأنماط الجينية المختلطة. أما بالنسبة للعينات الموجبة لحمض HCV النووي الريبي، فقد كان النمط الجيني 4 هو الأكثر انتشارًا، يليه النمط الجيني 1، مع نسبة ضئيلة من الإصابات المختلطة. تُظهر هذه النتائج أن عدوى التهاب الكبد B وC لا تزال منتشرة بين المرضى الذين يرتادون مرافق الرعاية الصحية في غرب ليبيا، مع كون التهاب الكبد B أكثر انتشارًا من التهاب الكبد C. يتوافق انتشار النمط الجيني D لفيروس التهاب الكبد B والنمط الجيني 4 لفيروس التهاب الكبد C مع الأنماط الوبائية الجزيئية الإقليمية، وله آثار هامة على الإدارة السريرية، واختيار العلاج، وتخطيط الصحة العامة. ويُعدّ استمرار المراقبة الجزيئية وتوسيع نطاق استراتيجيات الفحص أمراً ضرورياً لدعم الجهود الوطنية لمكافحة التهاب الكبد والقضاء عليه.