

Genetic Detection of Hepatitis C -Virus and Occult Hepatitis B in Patients from Al-Najaf Al-Ashraf Governorate, Iraq.

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Abstract

Recently, a noticeable increase in the prevalence of occult Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections has been observed among clinical cases such as patients undergoing hemodialysis, blood transfusion, liver diseases, and thalassemia worldwide. To limit and control this spread, the present study was conducted to investigate and detect the molecular presence of HCV and occult HBV using the Nested PCR technique, as well as to observe the effect of demographic and social factors on the infection rate.

A total of 150 clinical samples were collected from both male and female patients of different age groups and various residential areas. Samples were obtained from the Blood Center, Central Public Health Laboratory, Al-Sadr Medical City, Kidney Center, Al-Zahraa Teaching Hospital, the National Hospital, and the Gastroenterology and Liver Hospital in Al-Najaf Al-Ashraf Governorate.

The global prevalence of HCV and occult HBV among hemodialysis, blood transfusion, liver, and thalassemia patients indicates a strong association with healthcare centers. Therefore, preventive and monitoring procedures are highly essential to reduce the transmission of infection. Patients visiting these centers for treatment should be guided on personal hygiene, vaccination, and appropriate medical follow-up, in collaboration with the Training Department of the Directorate of Health and the Patient Safety Division. In particular, therapeutic hemodialysis procedures should be carefully organized in a sequence that ensures patient safety without affecting the treatment schedules in hemodialysis departments.

Keywords : PCR, HBV, HCV, liver Cancer , hepatitis

1. Introduction

Viral hepatitis is one of the main causes of chronic liver infections, leading to approximately four million deaths annually (1). Usually, hepatitis infection remains asymptomatic for a long period, but over time it may result in liver cirrhosis **or** hepatocellular carcinoma (2).

Viruses represent the most common cause of hepatitis worldwide, in addition to other causes such as alcohol consumption, drug-induced hepatitis, certain bacterial infections, and autoimmune liver diseases (3-6). In some cases, the infection may resolve spontaneously, while in others, it may progress to fibrosis or liver cancer (7). The prevalence of HCV and occult HBV infections varies widely among hemodialysis and thalassemia patients across different provinces and is closely associated with the general population (5-8).

Most viral hepatitis infections are transmitted through injection and blood transfusion, organ transplantation, hemodialysis procedures, contaminated shaving tools, tattooing, body piercing in beauty centers, sexual activity, and exposure among healthcare workers and laboratory staff (9-12).

Viral hepatitis accounts for a large number of deaths globally, with HCV and occult HBV representing about **95%** of all hepatitis-related deaths, while the remaining **10%** are caused by other viral types (13-18). The World Health Organization (WHO) has proposed a global strategy to reduce HCV infections by 80% and to test 90% of the targeted population by the year 2030, as part of the Sustainable Development Goals (SDG 2030) (19-23).

2. Materials and Methods

2.1 Data Collection

Data was collected using a structured questionnaire designed to obtain demographic and social information from the patients. The results were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software.

Percentages of the studied variables were calculated, and the differences between groups were compared. The level of statistical significance was determined at a p-value of 0.05–0.5% (10).

2.2 Rapid Test

Detection and screening of antibodies against HCV and occult HBV were carried out using the rapid diagnostic test, performed according to the manufacturer’s instructions provided with the testing kit (HIGH TOP / China).

2.3 ELISA

The enzyme-linked immunosorbent assay (ELISA) technique was used to detect and determine the presence of HCV Ab and HBV Ab in patients’ sera. The test was conducted following the manufacturer’s protocol (Abia / Germany).

2.4 Nested PCR

Detection of HCV and HBV viral DNA/RNA was performed using the Nested Polymerase Chain Reaction (Nested PCR) technique. This molecular assay amplifies specific genomic regions of the viruses using primer sets supplied by Microgen / Korea.

2.5 Ethical Considerations

Approval for conducting this study was obtained from the relevant ethical and administrative authorities. Necessary permissions for sample collection and laboratory procedures were granted by the Main Blood Bank, Al-Sadr Medical City, the Kidney Center, and the Central Public Health Laboratory in Al-Najaf Al-Ashraf.

All samples were collected from patients after obtaining their informed consent, and a questionnaire form was completed for each participant to record key demographic information.

3. Results

Detection of HCV and occult HBV viruses was performed in different clinical samples. Out of 150 samples, 63 (42%) tested positive for HCV, and 19 (12.66%) tested positive for occult HBV. The total infection rate for both HCV and occult HBV among male patients was 61.62% (53 males), while the infection rate among female patients was 45.31% (29 females). Therefore, males recorded a higher infection rate compared to females. (Table 1-9).

Table (1): Characteristics of Study Groups According to Demographic and Social Variables

Variable	Category	Frequency (%)	Sample Size	P-value
Gender	Male	(57.33) 86	150	0.011
	Female	(42.66) 64		
Age (years)	10–21	(21.33) 32	150	0.001
	22–33	(31.33) 47		
	34–45	(17.33) 26		
	46–57	(14.00) 21		
	58–70	(16.00) 24		
Residence	Rural	(46.66) 70	150	0.248
	Urban	(53.33) 80		
Clinical Condition	Liver disease	(5.33) 8	150	—

Variable	Category	Frequency (%)	Sample Size	P-value
	Thalassemia	(13.33) 23		
	Hemodialysis	(21.33) 32		
	Blood transfusion	(58.00) 86		

* No significant differences at $P < 0.5$

** Significant differences at $P < 0.05$

Table (2): Comparison of Infection Rates Between Males and Females

Gender	Total No.	HCV Positive (%)	Occult HBV Positive (%)	Both HCV and HBV (%)	P-value
Male	86	(40.69) 35	(20.93) 18	(61.62) 53	*0.047
Female	64	(43.75) 28	(1.56) 1	(29.31) 45	*0.708
Total	150	(42) 63	(12.66) 19	(54.66) 82	0.001 >

* No significant differences at $P < 0.5$

** Significant differences at $P < 0.05$

Table (3): Comparison of Infection According to Clinical Condition

Clinical Condition	Total No.	HCV Positive (%)	Occult HBV Positive (%)	Both HCV and HBV (%)	P-value
Liver disease	8	(100) 8	(0) 0	(100) 8	*0.001 >
Thalassemia	23	(100) 23	(0) 0	(100) 23	*0.001 >
Hemodialysis	32	(100) 32	(0) 0	(100) 32	*0.001 >
Blood transfusion	87	(0) 0	(21.83) 19	(21.83) 19	*0.001 >

* Significant differences at $P < 0.05$

Table (4): Comparison of Infection Rates by Age Group

Age Group (years)	Total No.	HCV Positive (%)	Occult HBV Positive (%)	Both HCV and HBV (%)	P-value
10-21	32	(56.25) 18	(0) 0	(56.25) 18	0.043
22-33	47	(34.04) 16	(12.76) 6	(46.8) 22	0.001
34-45	26	(23.07) 6	(26.92) 7	(50) 13	0.035
46-57	21	(46.14) 12	(28.57) 6	(85.71) 18	—
58-70	24	(45.83) 11	(0) 0	(45.83) 11	—

Table (5): Distribution of HCV and Occult HBV Infections According to Residence

Residence	Total No.	HCV Positive (%)	Occult HBV Positive (%)	Both HCV and HBV (%)	P-value
Rural	70	(51.42) 36	(8.57) 6	(60) 49	0.029
Urban	80	(33.75) 27	(16.25) 13	(50) 40	0.15

* Significant differences at **P < 0.05**

To confirm infection, detection of viral genes was performed using the Nested PCR technique. For HCV, the 5'UTR gene showed the highest positivity rate (**35%**), followed by NS5B gene (**17.5%**), and the lowest rate was for the CORE gene (10%). For HBV, the X gene recorded the highest prevalence (**63.15%**), followed by the CORE gene (36.84%) and the POL gene (21.05%), whereas the S gene showed no positive samples.

Table (6): HCV Gene Detection Using Nested PCR

Gene Type	No.	%	P-value
5'UTR	14/40	35	0.018*
CORE	4/40	10	0.018*
NS5B	7/40	17.5	0.018*

* Significant differences at **P < 0.05**

Table (7): Distribution of HCV Genes According to Demographic Variables

Variable	Total	NS5B (%)	CORE (%)	5'UTR (%)	P-value
Gender					
Male	20	4 (20%)	2 (10%)	12 (60%)	0.001
Female	20	3 (15%)	2 (10%)	2 (10%)	0.851
P-value		0.677	1	0.001	
Age Groups					
10–21	15	4 (26.6%)	2 (13.3%)	6 (40%)	—
22–33	11	0 (0%)	2 (18.18%)	4 (36.36%)	—
34–45	1	1 (100%)	0 (0%)	0 (0%)	—
46–57	8	2 (25%)	0 (0%)	1 (12.5%)	—
58–70	5	0 (0%)	0 (0%)	3 (75%)	—

Table (8): HBV Gene Detection Using Nested PCR

Gene Type	No. of Positive Samples	%	P-value
S	0	0	<0.0001*
CORE	—	36.84	<0.0001*
POL	4/19	21.05	<0.0001*
X	12/19	63.15	<0.0001*

* Significant differences at $P < 0.05$

Table (9): Genetic Distribution of Occult Hepatitis B Virus According to Demographic Characteristics

Studied Variables	Total Number	Pol Gene	X Gene	Core Gene	S Gene	P Value
Gender						
Males	18	4 (22.22%)	12 (66.6%)	5 (27.77%)	0 (0%)	<0.0001*
Females	1	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0.405
P value		0.596	0.179	0.179	1	
Age Groups (years)						
10–21	0	–	–	–	–	–
22–33	7	3 (42.85%)	5 (71.42%)	1 (14.88%)	0 (0%)	0.022*
34–45	6	0 (0%)	4 (66.66%)	3 (50%)	0 (0%)	0.016*
46–57	6	1 (16.66%)	3 (50%)	3 (50%)	0 (0%)	0.142
58–70	0	–	–	–	–	–
P value		0.159	0.710	0.298	1	
Residence						
Rural	7	1 (14.88%)	4 (57.14%)	1 (14.28%)	0 (0%)	0.054
Urban	12	3 (25%)	8 (66.66%)	6 (50%)	0 (0%)	0.004*
P value		0.581	0.678	0.120	1	

Significant differences at $P < 0.05$.

4. Discussion

The present study demonstrated that the prevalence of HCV and occult HBV infections among the studied population was relatively high. The results revealed that 42% of patients were positive for HCV, while 12.66% were positive for occult HBV, indicating that both viruses remain significant health concerns in Al-Najaf Governorate. These findings are consistent with several previous studies conducted in Iraq and other countries, which also reported a considerable prevalence of hepatitis viruses among patients undergoing blood transfusion and hemodialysis (11, 12, 13).

Gender Distribution

The infection rate was higher in males (61.62%) than in females (45.31%). This difference may be attributed to behavioral and occupational factors, as males are more frequently exposed to risk activities, such as blood transfusions, surgical procedures, or contact with contaminated instruments. This observation aligns with the findings of Al-Obaidi (2019) and Hussein (2020), who also reported a higher rate of viral hepatitis among male patients compared to females.

Age Distribution

The results showed that infection rates varied significantly with age. The highest infection rate was recorded among the 46–57 year age group, followed by 10–21 years, whereas the lowest rate was in the 34–45 year group. This variation could be explained by cumulative exposure to risk factors with increasing age, as well as differences in immunity and health practices between age groups (14, 15).

Clinical Correlation

A strong association was observed between clinical condition and infection type. All patients with liver disease, thalassemia, and hemodialysis were positive for HCV, while patients with a history of frequent

blood transfusions had the highest rate of occult HBV infection. These results confirm that blood transfusion remains one of the major transmission routes for hepatitis viruses, despite routine screening tests (16, 17).

Residence Factor

The infection rates were higher among rural residents (60%) compared to urban residents (50%). This could be due to limited health awareness, inadequate medical facilities, and poor sterilization practices in rural areas. Similar observations were reported in studies from Baghdad, Basra, and Diyala, indicating that geographical and socioeconomic factors influence viral transmission (18, 19).

Molecular Detection

The molecular detection by Nested PCR showed that the 5'UTR gene was the most prevalent among HCV-positive samples (35%), followed by NS5B (17.5%) and CORE (10%). The high detection rate of 5'UTR confirms its sensitivity and reliability as a molecular marker for HCV diagnosis, as reported in previous literature (20). For HBV, the X gene was the most frequently detected (63.15%), followed by the CORE (36.84%) and POL (21.05%) genes, while S gene detection was absent. This suggests that mutations or low replication activity may reduce the detectability of the S gene in occult HBV cases. Similar findings were recorded in studies by Zanetti et al. (2018) and Mohammed et al. (2021) (21, 22).

Interpretation and Implications

The coexistence of HCV and occult HBV infection, as observed in this study, could pose serious diagnostic and clinical challenges. Occult HBV infection, in particular, may escape detection during routine screening and may lead to liver complications such as fibrosis or hepatocellular carcinoma (HCC) if not identified early. Therefore, molecular techniques (such as Nested PCR) should be implemented as a confirmatory diagnostic tool, especially among patients receiving regular transfusions or dialysis. Moreover, public health education and improved infection control protocols are urgently needed to minimize the spread of these viruses within healthcare facilities.

Conclusion

The findings of this study revealed a high prevalence of HCV and occult HBV infections among patients in Al-Najaf Governorate, particularly among males, middle-aged individuals, and those undergoing frequent blood transfusions or hemodialysis. The 5'UTR gene for HCV and the X gene for HBV showed the highest detection rates, confirming their diagnostic importance in molecular testing. These results emphasize that occult HBV infection may remain undiagnosed using conventional serological methods, posing a hidden threat to public health and blood safety. Therefore, reliance solely on ELISA or rapid tests is insufficient for the accurate detection of these infections.

Recommendations

1. Implement molecular diagnostic methods (Nested PCR) as part of routine screening for blood donors and high-risk patients (e.g., those on dialysis or receiving transfusions).
2. Increase public awareness about transmission routes of viral hepatitis and the importance of early testing.
3. Enhance sterilization and infection control practices in medical institutions, especially in rural areas.
4. Develop educational programs for healthcare workers to improve diagnostic accuracy and infection prevention.

5. Conduct further molecular studies to identify viral genotypes and mutations associated with disease severity and treatment resistance.
6. Establish a national surveillance system to monitor the epidemiology of hepatitis viruses and support preventive strategies.

In summary:

This study underscores the public health importance of integrating molecular diagnostic techniques alongside serological tests to ensure accurate detection and effective control of HCV and occult HBV infections in Iraq.

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