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Identification and Diagnosis of Active and Inactive Hepatitis B Virus among Foreign-Resident Individuals Using Real-Time PCR-Based Assays in Erbil Governorate, Iraq

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Ayad Kareem Ali [1*]

Abstract

Background and Objective: Real-time Polymerase Chain Reaction's main advantages lie in its high sensitivity and its capacity to develop rapid assays. In regions with high foreign residents, such as Erbil Governorate, effective diagnosis and differentiation of active and inactive HBV infections are of paramount importance for public health management. The present study aims to determine the prevalence of HBV infection among foreign-resident based on Real-time PCR results and providing insights that may have implications for public health strategies.

Methods: In this study, a Real-time PCR was used to detect HBV DNA among 193 foreign -resident individuals with hepatitis B.

Results: Among the clinically diagnosed individuals at the Erbil public health laboratory, a study encompassed 193 HBsAg-positive foreign-resident individuals, comprising 107 males and 86 females, aged between 23 and 47 years. Among males, 60 (56.74%) of them had HBV DNA of (≥ 3.8 IU/ml), whereas among females, 48 (55.8%) of them exhibited HBV DNA of (≥ 3.8 IU/ml). The study is conducted on foreign-resident individuals from seven different nationalities. The minimum viral load was observed among Chinese (0 IU/ml), while the maximum viral load was recorded among Syrians (2,398,805, 400 IU/ml) according to sample size. The study found that among ELISA-positive individuals, 44.04% were categorized as having inactive HBV infections; in contrast, 55.95% of the individuals were classified as active carriers.

Conclusion: Understanding the distribution of HBV infection and their viral loads across different resident nationalities in Erbil governorate is essential for controlling the spread of HBV and tailoring prevention and treatment programs. Detection and quantification of HBV DNA are crucial in diagnosis and monitoring HBV infection and evaluating therapeutic responses.

Keywords: HBV DNA; Foreign residents; Real-Time PCR; Erbil; Iraq.

Introduction

Hepatitis B is an actually life-threatening liver infection caused by hepatitis B virus (HBV).[1] The virus is a partially double-stranded DNA virus, part of the Hepadnaviridae family (Figure 1). It is a significant contributor to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma.[2] The worldwide prevalence of chronic hepatitis B virus infection is approximately 257–291million individuals.[3] Chronic hepatitis B disease exhibits a wide range of manifestations, from inactive carriers to individuals with chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). According to the World Health Organization (WHO), hepatitis B was responsible for an estimated 887,000 deaths in 2015, largely due to cirrhosis and HCC.[4]

Some research in Iraq indicated the prevalence of HBV infection as between 0.7% and 1.37%.[5,6] In a prior study conducted in Northern Iraq's Duhok city,

[1] Department of Midwifery, Koya Technical Institute, Erbil Polytechnic University, Erbil, Iraq.
Correspondence: ayad.ali@epu.edu.iq

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the HBV prevalence among blood donors was identified as 1.14%.[7] Findings from Erbil city (in northern Iraq) showed that the seropositivity of occult hepatitis B infection (OBI) was 39.1%.[8] Despite the significant reduction in new cases due to the universal HBV vaccine, HBV infection remains a considerable public health concern in Saudi Arabia, exhibiting a prevalence of 1.9% in men and 1.43% in women.[9]

The determination of HBV infection epidemiology primarily relies on detecting the hepatitis B surface antigen (HBsAg) within the general population. This method categorizes the geographic prevalence of HBV infection into three endemicity regions: high (>8%, East Asia, Africa), medium (2–8%, Mediterranean, Eastern Europe), and low (<2%, North America, Western Europe) endemicity.[10] Real-time PCR has emerged as the leading diagnostic technology for detecting various viruses among nucleic acid amplification techniques. Due to its high sensitivity and broad dynamic range, Real-time PCR is progressively replacing other signal and target amplification methods for HBV DNA detection.[11-13]

Among patients with undetectable HBV DNA, the presence of hepatitis B core antigen (HBcAg) indicates continued transcription of covalently closed circular DNA (cccDNA). This discovery can predict clinical relapse and assist clinicians in identifying patients at a higher risk of developing hepatocellular carcinoma (HCC).[14,15] The diagnosis of chronic HBV infection relies on the detection of HBV serologic markers, including hepatitis B surface antigen (HBsAg), hepatitis B core antibodies, or the identification of HBV DNA through molecular assays.[16] Detectable HBV DNA in serum or plasma serves as a reliable marker of active HBV replication, confirming infectivity. While the diagnosis of acute and chronic HBV infection typically involves serologic methods, measuring HBV DNA serum levels is crucial for diagnosing the infection phase, determining the need for treatment, and subsequent patient monitoring.[16,17] The fact that the Real-time PCR platform is a multipurpose platform and can be applied in various fields of application is worthy of exploration. The technique can be used for basic molecular research right through to an approved molecular diagnostic assay. The exploration of the current wide range of applications of the Real-time PCR method is critical, including its feasibility in low-middle income countries.[18] In this study the quantification of viral load was performed using (RT-PCR) testing at the Erbil Public Health Laboratory. Specifically, the study focused on measuring viral load levels to distinguish between active and inactive HBV cases among foreign-resident individuals in Erbil Governorate.

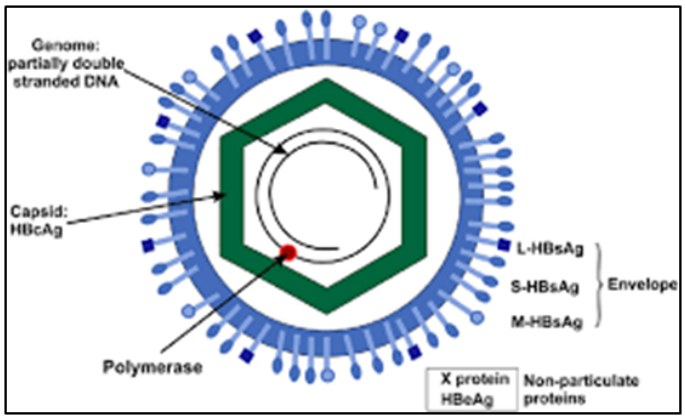

Figure 1 Schematic representation of hepatitis B virus (HBV), showing the structure of the virion, composed of a partially double stranded DNA genome, enclosed by a capsid

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Methods

Study Design and Patient Selection

• A cross-sectional study was carried out at the Erbil Public Health Laboratory from January to August 2023.

• In the current study, a total of 193 foreign-resident individuals who had been serologically diagnosed as HBsAg-positive (among 300 tested individuals diagnosed with both positive and negative for enzyme-linked immunosorbent assay results) and were suspected of having viral hepatitis based on clinical symptoms were included.

•The patients were referred for HBV DNA screening and follow-up assessment of viral load.

Sample Collection and Processing

• Blood samples were collected from clinically diagnosed HBsAg positive individuals at the Erbil Public Health Laboratory.

• Five milliliter's of venous blood were collected from each person using gel tubes. Furthermore the samples labeled and centrifuged at 4000xg for 10 min and the Viral HBV DNA was extracted from a 200µL aliquot of serum using a Qiagenmini blood kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Viral Load Estimation

• The quantification of HBV DNA was performed using Real-time PCR (HBV RG PCR ArtusGermany) Figure 2.

• For the Real-time PCR reaction, a 50µL reaction mixture was prepared, consisting of 30µL of Master-mix (Buffer, dNTP, primer, probe, and enzymes) and HBV RG inhibition control mixed with 20 µL of DNA template.

• Specific primers were used for the real-time PCR reaction: HBV-Taq I forward primer (CAA CCT CCA ATC ACT CAC CAA) and HBV-Taq 2 reverse primer (ATA TGA TAA AAC GCC GCA GAC AC).[19] (Figure 2)

Rotorgene 3000 was employed for the Real-time PCR process to detect HBV-DNA, following the manufacturer's instructions. The Real-time PCR cycling The quantitation of HBV DNA was performed for all blood samples, and results were considered positive and significant when the viral load was equal to or greater than 3.8 IU/ ml.[20] Titers less than 3.8 IU/ml were classified as negative for HBV DNA. The screening results were categorized based on positive HBV DNA titers into two groups: inactive and active carriers.

Statistical analysis: The collected data were transferred to an Excel spreadsheet. Descriptive statistics of the variables were analyzed and are presented in the form of tables and graphs. The results were statistically evaluated using Microsoft Excel Office 2010.Mean and standard deviation for the viral loads for both genders and each nationality are calculated using equation (1) and equation (2).

Mean

$$\text{Mean} = \frac{\sum \text{viral load values}}{\text{number of values}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Standard Deviation

$$SD = \sqrt{\frac{\sum(x_i - \text{mean})^2}{n-1}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Figure 2 Qiagen, Real Time-PCR Machine

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Ethical Approval

Ethical approval for this study was granted by the Medical Ethics Committee of the Koya Technical Institute/ Erbil Polytechnic University (approval number 11198, dated November 12, 2023). An official acceptance letter was obtained from the Erbil Public Health Laboratory, authorizing the research to be conducted at the laboratory. All participants were assured that their confidentiality would be maintained and that their information would only be used for research purposes.

Results

This study aimed to evaluate the effectiveness of viral load detection as a diagnostic test for HBV infection in Erbil Governorate. The quantification of viral load to determine active and inactive HBV was performed using Real-time PCR. Out of a total of 300 individuals with positive and negative ELISA HBsAg results at the Erbil Public Health Laboratory, 193 serologically diagnosed foreign-resident individuals with HBsAg positivity were included in the study, ranging in age from 23 to 47 years. The study encompassed individuals from seven distinct nationalities - Syrians, Turks, Iranians, Egyptians, Bangladeshis, Indians, and Chinese—each linked to diverse geographic regions. They visited the Erbil Public Health Laboratory for testing.

Table 1 presents data on the Hepatitis B Virus (HBV) detection results in foreign-resident individuals, stratified by gender (Male and Female). A total of 107 males and 86 females were subjected to testing. Among males, 60 individuals (accounting for 56.74% of the male population) had HBV DNA detected (≥ 3.8 IU/ml), whereas among females, 48 individuals (constituting 55.8% of the female group) exhibited HBV DNA presence (≥ 3.8 IU/ml). Conversely, some ELISA-positive individuals registered as "not detected" in the Real-time PCR test results, indicating viral loads below 3.8 IU/ml. Specifically, 47 males (43.9% of males) fell into this category, and 38 females (44.18% of females) had their HBV DNA not detected (< 3.8 IU/ml). The minimum viral load for males was 123 IU/ml, while for females, it was 300 IU/ml. In contrast, the maximum viral load observed among males was 424,482,600 IU/ml, and among females, it reached 2,398,805,400 IU/ml. The mean and standard deviation for males was 32.36667± 30.95745, and for females, it was 29.4375 ± 27.97368421, respectively.

Table 1 Hepatitis B Virus (HBV) DNA detection by gender and viral load levels with Mean ± SD for each gender

Sex	Detected (≥ 3.8 IU/ml)	Not Detected (< 3.8IU/ml)	Total	Minimum Viral Load	Maximum Viral Load	Mean ± SD
Male	60 (56.74%)	47 (43.9%)	107	123 IU/ml	424,482.600 IU/ml	32.36667 ± 30.95745
Female	48 (55.8%)	38 (44.18%)	86	300 IU/ml	2,398,805,400 IU/ml	29.4375 ± 27.97368421

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Table 2 provides the results of Hepatitis B Virus (HBV) testing among individuals from various nationalities belonging to different geographic area.

Table 2 and Figure 3 include data on the number of individuals with detected Real-time PCR results: Syrians (36), Turks 32), Iranians (17), Bangladeshis (16), (Egyptians (4), Indians (3), and Chinese (0). It also includes individuals with not detected results: Syrians (24), Turks (28), Iranians (15), Bangladeshis (10), Egyptians (3), Indians (1), and Chinese (4). Table 2 also shows the mean ± SD for each nationality.

Table 2 Nationality-Based HBV Hepatitis Viral Load Detection and Statistical Analysis

Nationality	detected	Not detected	Total No. (%)	Mean ± SD
Syrian	36	24	60 (31.08)	30.18421053 ± 31.18182
Turks	32	28	60 (31.08)	33.53167 ± 27.75
Iranian	17	15	32 (16.5)	31± 28.72727
Bangladeshi	16	10	26 (13.4)	33.5 ± 30.4444444
Egyptian	4	3	7 (3.6)	29.5 ± 32.66666667
Indian	3	1	4 (2.07)	32.666667± 43
Chinese	0	4	4 (2.07)	-
Total	108	85	193	

*Detected indicating presence of HBV in PCR. Not detected indicating an absence of HBV in PCR.

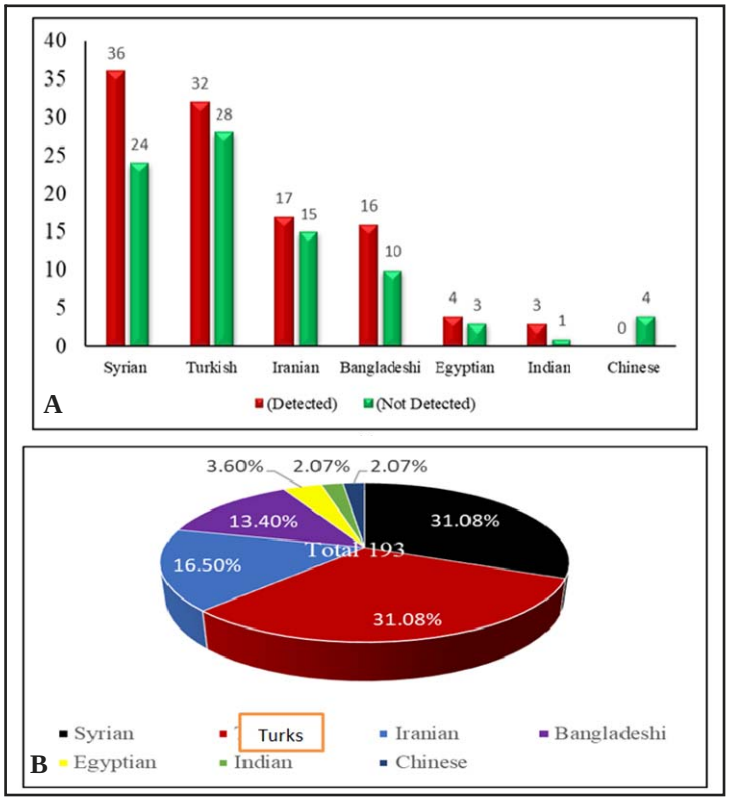

Figure 3 (a) Frequencies of detected and not detected Hepatitis B Virus (HBV) in PCR and **(b)** Infection rate among different resident nationalities

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Among the ELISA-positive blood samples analyzed in Table 3, the discrimination between active and inactive HBV infections in HBV-DNA blood samples relied on to HBV-DNA titers. Consequently, individuals were classified into inactive carriers (≤ 3.8 IU/ml) and active carriers (> 3.8 IU/ml). The data presented showed that 44.04% of individuals had inactive infections, while 55.95% were categorized as active carriers.

Figure 4 demonstrates a typical Real-time PCR amplification curve by plotting the quantity of PCR product (amplicon) against the number of reaction cycles. The Figure composed of multiple panels, including top panels (quantitation analysis graphs), middle panels (quantitation results tables), and bottom panels (standard curve graphs) for two different dyes: Cycling A.Green and Cycling A.Yellow.

The number of DNA molecules present in the starting mixture determines the quantity of amplicon generated after a set number of PCR cycles. If the starting mixture contains only a few DNA molecules, relatively little amplicon will be synthesized, and the curve will fall. Conversely, if there are large amounts of starting molecules, the amount of product will be higher, and the curve will rise. Plotting the amount of PCR product (amplicon) versus the number of reaction cycles results in a representative real-time PCR amplification curve, as illustrated in Figure 4.

Table 3 Active and inactive HBV infection percentages among foreign individuals, categorized according to titers

Inactive carriers	Active carriers
≤ 3.8 IU/ml	> 3.8 IU/ml
85 (44.04%)	108 (55.95%)

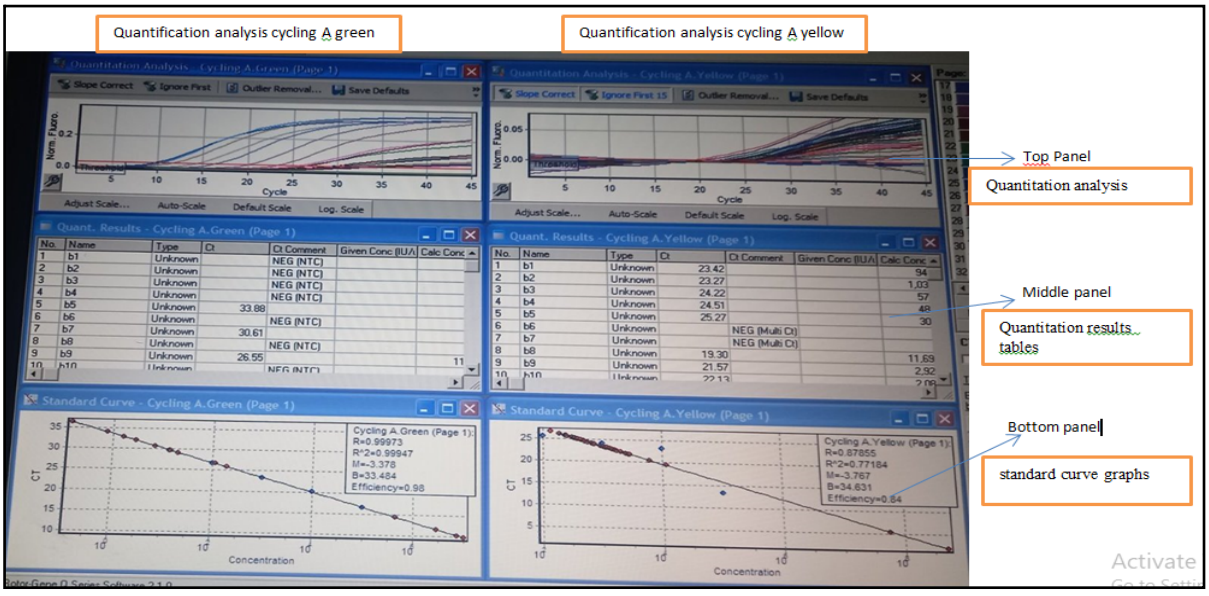

Figure 4 Quantitative PCR (qPCR) analysis software graph showing quantification and amplification curves of Hepatitis B Virus (HBV) DNA

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Discussion

Hepatitis B virus (HBV) is responsible for causing both acute and chronic infections. Chronic hepatitis B (CHB), in particular, poses a significant public health challenge, currently affecting over 257 million individuals worldwide.[21]

Both male and female individuals were included (107 males and 86 females). The age range of the participants was between 23 and 47 years. The data presented in Table 1 provides insights into Hepatitis B Virus (HBV) DNA detection rates and viral load levels by gender. Among the total participants, males exhibit a slightly higher detection rate of 56.74% [HBV DNA (≥ 3.8 IU/ml)] compared to females, who have a detection rate of 55.8% [HBV DNA (≥ 3.8 IU/ml)]. This indicates that the prevalence of HBV DNA is relatively similar between genders, with males having a marginally higher rate of detected cases. The results of another study by Amer *et al.* in (2020) that the gender positivity for PCR test, in total of 154 male patients 65 (42%) were positive and of 102 female patients only 28 (27%) were positive.[22]

Conversely, some individuals who initially tested positive for the ELISA method were later identified as "not detected" based on the PCR test results, indicating that their viral loads fell below the 3.8 IU/ml threshold. Specifically, 47 males (out of 107), representing 43.9% of the male population, were categorized as "not detected". Similarly, 38 females (out of 86), making up 44.18% of the female group, had their HBV DNA levels fall below the 3.8 IU/ml threshold. Previous study achieved in (2019) by Essam *et al.* showed that the overall HBV prevalence among males' patients to be 58.5%, while it as 41.5% among females. The findings revealed a considerably higher risk of HBV infection among males compared to females.[23]

The analysis of viral load reveals that males have a minimum viral load of 123 IU/ml and a maximum viral load of 424,482,600 IU/ml, with a mean ± SD of 32.37 ± 30.96. In contrast, females display a wider range of viral loads, with a minimum of 300 IU/ml and a maximum reaching as high as 2,398,805,400 IU/ml. Their mean viral load stands at 29.44 ± 27.97.In a prior study by Huda which involved 105 (65 males and 40 females) ELISA (HBsAg) positive outpatients, 72 (60.5%) were positive for HBV DNA via the first PCR, while 33 (31.4%) tested negative for the presence of HBV DNA.[24]

Among individuals who tested positive in ELISA at Erbil Public Health Laboratory, only 32% were local, while the remaining 68% were non-local. Inversely an earlier study highlighted a higher prevalence of HBV infection among locals (1.6%) in various areas of Iraq compared to incoming individuals (0.6%).[24] The lower HBV positivity among local residents in Erbil compared to non-locals, and the inverse trend observed in other areas of Iraq, likely reflects a complex interplay of migration, healthcare access, public health initiatives, and socioeconomic factors. Erbil's status as a major urban center with a large migrant population and a potentially stronger healthcare system may explain the observed differences in HBV prevalence. The data show significant differences in HBV detection rates among individuals from different nationalities. Syrians and Turks comprised the largest portion of the sample, with these groups exhibiting relatively high mean viral loads, indicating a substantial presence of HBV within these populations. This result aligns with a study that demonstrated the prevalence of HBV among Syrian refugees is approximately four times higher than in the native Iraqi population.[25] Additionally, a previous study conducted in Turkey, a neighboring country of Iraq, revealed that the prevalence of HBV positivity varied significantly, ranging from 1% to 14.3%, depending on the geographical region and the characteristics of the recruited samples.[26]

Non-local residents, particularly migrants and refugees may have higher HBV infection rates due to disrupted healthcare

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access, inadequate vaccination programs, and socioeconomic challenges. These factors make them more vulnerable to HBV infection, with many potentially contracting the virus before arriving in Erbil.

Iranians and Bangladeshis showed comparable detection rates with 17 out of 32 (53.125%) Iranians and 16 out of 26 (61.5%) Bangladesh is testing positive. Despite having a smaller sample size, these groups had a slightly higher detection rate compared to Syrians and Turks, indicating a considerable prevalence of HBV. According to the results of a study the prevalence of HBV infections in the Iranian population is 1.7%.[27] In Bangladesh continuous approaches by the government, private sector, non-governmental organizations and various organizations have been contributing to improving the situation to reach the 2030 hepatitis elimination target.[28] As a result, from the recent government report and studies (2018–2022), the prevalence of HBV was 4–4.5% and that of HCV as 0.5–0.6% in the general population of Bangladesh.[28,29]

Egyptians and Indians had lower representation in the study, with detection rates of 4 out of 7 (57.1%) and 3 out of 4 (75%), respectively. While these groups demonstrated varied detection rates, their mean viral loads were consistent with the observed detection trends.

Chinese participants displayed the unique outcome of having no detected cases among the four individuals sampled, highlighting a potential variance in HBV prevalence compared to other nationalities.

The study also highlights significant diversity in the mean viral loads among the detected cases. The mean viral loads, expressed as Mean ± SD, highlight the variability in HBV presence within each group Table 2. The Turks showed the highest mean viral load (33.53 ± 27.75), suggesting a higher concentration of HBV among detected cases. Conversely, Egyptians demonstrated the lowest mean viral load (29.5 ± 32.67), although the high standard deviation indicates significant variability among positive cases. The standard deviations across groups reveal considerable fluctuations in viral load levels, with Indians exhibiting the largest variability (32.67 ± 43), possibly due to the small sample size and individual differences. The results of this study show diverse HBV detection rates among various nationalities, with the largest portions of detected cases being among Syrians and Turks. The significant variance in mean viral loads and standard deviations across nationalities suggests that further studies may be necessary to understand the factors contributing to these disparities, such as genetic predispositions, healthcare access, and socio-environmental influences. The absence of detected HBV cases among the Chinese participants is particularly notable and warrants further investigation to determine if this finding is consistent in larger samples.[30]proved that real-time PCR was essential to precisely quantify viral particles per milliliters of blood and confirm HBV infection.

In this particular study the selected foreign-resident individuals were classified based on the titers of HBV-DNA in their blood samples, allowing differentiation between active and inactive HBV infections. The study found that among ELISA-positive individuals, 44.04% were categorized as having inactive HBV infections, with titers less than or equal to 3.8 IU/ml. In contrast, 55.95% of the individuals were classified as active carriers, having titers exceeding 3.8 IU/ml. The data reveals that a significant majority of the ELISA-positive individuals exhibited active HBV infections, indicating a higher viral load and potentially greater infectivity. The range of measured HBV levels is extensive, spanning from individuals with relatively low viral loads (123) IU/ml. to those with exceptionally high concentrations (2398805400) IU/ml. Another study In Basra governorate revealed that the majority of patients

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belonged to the inactive HBsAg carrier group, accounting for 54.76%.[31] The study's strengths include its use of real-time PCR, which offers high sensitivity and rapid detection of HBV DNA, making it a crucial tool for accurate diagnosis and monitoring of HBV infections. Additionally, by including foreign-resident individuals from seven different nationalities, the study provides valuable insights into the prevalence and distribution of HBV infection across diverse groups in Erbil Governorate. The findings also have significant public health implications, highlighting the need for targeted vaccination and treatment programs tailored to the distribution of HBV infection among various nationalities. The study's weaknesses include its relatively small sample size (193 individuals), which may limit the generalizability of the findings. A larger sample could yield more robust data and strengthen the conclusions. Additionally, the study does not thoroughly explore social factors such as living conditions, healthcare access, and cultural practices, which could influence HBV infection rates among different nationalities. Moreover, the study does not consider the occupations of participants, an important factor that could impact HBV transmission and prevalence, as certain jobs may carry higher risks of exposure to the virus.

Conclusion

Real-time PCR's ability to detect HBV DNA makes it an invaluable tool in the management and treatment of hepatitis B. By accurately measuring the viral load, healthcare providers can better assess the risk of liver damage, monitor treatment responses, and predict the potential for disease progression. Accurate differentiation between active and inactive HBV infections is crucial for several reasons. This differentiation helps reduce the burden on healthcare resources and minimizes unnecessary interventions. These findings emphasize the importance of distinguishing between active and inactive carriers in the context of HBV infections. Understanding the distribution of HBV infections and their viral loads across different resident nationalities in Erbil governorate is crucial for controlling the spread of HBV and tailoring prevention, vaccination, and treatment programs. Countries with higher detection rates and viral loads may need more extensive intervention efforts to reduce the burden of the disease.

Competing interests

The author declares that he has no competing interests.

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