

Prevalence of Subclinical Cases of HBsAg and IgM-HCV Positivity among Healthy Iraqi Individuals

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ABSTRACT

Background: A significant proportion of the world's population suffers from chronic viral hepatitis B (HBV) and hepatitis C (HCV) infection, and both HBV and HCV are leading causes of chronic liver disease (CLD), leading to significant morbidity and mortality.

Objective: This study aimed to understand the prevalence of subclinical cases of HBV-surface antigen (HBsAg) and IgM-HCV positivity among healthy individuals for early intervention and treatment through an enzyme-linked immune-sorbent assay test.

Materials and methods: A total of 250 blood samples were collected from healthy individuals (HI) along with surgeries, before marriage, and blood donors.

Results: showed that a reaction for HBsAg and IgM-HCV positivity was 16.4% (41/250) and 13.6% (34/250) of healthy individuals respectively as a sub-clinical case, and there was significant disappearance of the viral infection among HI. A higher frequency of viral occurrence was reported in ages between 21 to 40 years old. There was a significant increase in males 53.3% and 64.7% than in females 46.7% and 35.3% regarding HBsAg and IgM-HCV respectively. Furthermore, this study showed that there was significant HCV infection appearance in males than in females in contrast with HBsAg was not significant. There was a higher significant increase of the viral infection obtained among married ($p < 0.001$) females and males. This finding pointed out the reveal of viral infection mostly in individuals aged more than 30 years old accompanied by an increase with sexual contact.

Conclusion: Screening protocols being mandatory in government agencies for at-risk populations can help identify subclinical cases and prevent further transmission.

Keywords: Sub-clinical case, HCV-IgM, HBsAg, ELISA.

INTRODUCTION

A significant proportion of the world's population suffers from chronic viral hepatitis B (HBV) and hepatitis C (HCV) infection, and both HBV and HCV are leading causes of chronic liver disease (CLD), leading to significant morbidity and mortality⁽¹⁾. Unfortunately, due to vague and generic initial disease symptoms and asymptomatic progression into advanced-stage liver diseases, many patients are detected incidentally on routine laboratory investigations (RLI) and hepatic ultrasound⁽²⁾.

However, in areas like Asia, where CLD due to HBV is highly prevalent, it is yet not possible to estimate the actual magnitude of this problem, and most familiarly, patients may present with serological charades of a dual infection. It is of prime importance to affirm the viral nature of the etiology of CLD to slave the advancing liver fibrosis, i.e., the control of the progression of liver disease by anti-viral therapy to restore viral clearance. It is recently reported, that with the falling prevalence of active carriers of HBsAg, there is a spurt in the incidence of isolated anti-HBc IgG-positive CLD patients⁽³⁾.

Viral hepatitis B and C are serious public health problems, with a combined global prevalence of over 500 million. Approximately one-third of the world's population has been infected with HBV, and more than 350 million are chronic carriers⁽⁴⁾, which pose the risk of

developing complications such as CLD, liver failure, or cancer. HCV is a significant stealth virus that does not induce a potent immune response of adequate intensity and duration to eliminate the infection of the host organism⁽⁵⁾.

Globally, there are an estimated 71 million persons infected with HCV who are at risk for developing complications such as liver cirrhosis and hepatocellular carcinoma. Approximately 250,000 persons die each year in India due to HCV-related cirrhosis, NLH, and HCC. Hepatitis is a relatively common and serious disease both in developed and developing countries⁽⁶⁾.

It finds its way using unhealthy eating practices with the use of shared utensils, through dirty needles and syringes, and sometimes by using unsterilized instruments in dentistry or grooming. The disease of hepatitis has multiple forms such as type A, B, C, D, and E but type B and C versions prevail and are the cause of worry among people due to chronic infections that destroy the liver cells and lead to liver cancer⁽³⁾.

Hepatitis B disease remains the first on the list of public health important problems in Iraq. Despite the hepatitis B vaccines which are globally accessible, outbreaks still occur because the vaccination rates of adults are often low⁽⁷⁾. WHO projected that there were more than 350 million hepatitis B carriers around the world⁽⁸⁾. Efforts to fight HBV are of utmost importance:

interrupting the way of this infection transmission, treatment of chronically infected patients, and universal infant vaccination as the most effective ways to stop HBV spread. While there are no specific treatments available for acute infection, chronic cases can be managed with medications. Studying hepatitis C in healthy individuals is crucial for early detection, timely intervention, and effective management of the infection. This includes understanding prevalence rates across different demographics and implementing targeted screening programs to address this public health challenge ⁽⁹⁾.

In our population-based sero-surveys is necessary to detect the true prevalence of HBV and HCV infections across different territories and develop comprehensive screening strategies targeting high-risk groups. Thus, this study aimed to understand the prevalence of subclinical cases of HBsAg and IgM-HCV positivity among healthy individuals for early intervention and treatment. Early detection through ELISA test, is critical in preventing serious liver damage and improving long-term health outcomes.

MATERIALS AND METHODS

1- Study population: A total of 250 blood samples were collected from healthy individuals along with surgeries, before marriage, and blood donors, during the period from Oct. 2023 to Feb. 2024 from Baghdad hospitals and private laboratories. These participants were selected randomly with different ages from 12-68 years (33±15.3) (95 females and 155 males). These samples were tested for HBsAg and Hepatitis C antibodies (IgM) as a sub-clinical case. A detailed case investigation was filled up for each one of them regarding their socioeconomic status, age, sex, clinical signs, and history of hepatitis infection.

2-Blood samples collection: Blood samples (3 ml) were collected by disposable syringe and then placed into a plane tube which stood at room temperature until coagulation. Then, the samples were centrifuged at 3000 rpm for 5 minutes. Serum samples were dispensed separately on Eppendorf tubes.

3-Detection for Hepatitis B surface antigen and IgM-HCV antibodies: All samples were stored at -20 °C till tested. The tests were done using an Enzyme-Linked Immunosorbent Assay specific for HBsAg and HCV-IgM-ELISA (Biotech; HONGKONG) kit reagents for the qualitative detection of HBsAg and antibodies IgM to Hepatitis C virus in humans, following the manufacturer's instructions. The results were read by a microwell reader and compared in a parallel manner with the calibrator and controls.

Quality control: The following criteria were used for the positive reactions of each test and sample depending on the kit company. The optical density value of the reagent blank against air from a microwell reader was less than 0.120; meaning that the Cut-off value was +0.12.

Interpretation: According to the supplying company instructions; positive results sample OD value was \geq C.O. This means that the given specimen absorbance was equal to or greater than the cut-off value. The negative results sample OD value was $<$ C.O. This means that the given specimen absorbance was less than Cut-off value.

Ethical approval: The Ethics Committee of Public Health and The Environment and Department of Biology at College of Science, University of Baghdad approved the study (CSEC/0923/0059). The Helsinki Declaration was followed throughout the study's conduction.

Statistical Analysis

The data were analyzed using SPSS IBM version 25 IBM. The proportion and their frequencies were checked by applying the Person Chi-square test to investigate significant comparisons between percentages of viral infection. One-sample T-test was used to calculate the significance, mean, and standard deviation of participants' ages. The P-values \leq 0.05 are considered statistically significant.

RESULTS

Prevalence of Subclinical HBsAg Positivity:

Results showed that 16.4% (41/250) of healthy individuals (HI) had a positive HBsAg as a sub-clinical case, and there were significant disappearance of the HBsAg among HI (Chi-square= 196.8, $p <$ 0.001). A higher frequency of viral occurrence was reported in ages between 21 to 40 years old, but approximately equal percentage between males 53.3%, and females 46.7%. Moreover, a significant difference was observed among study age groups as summarized in table (1).

Table (1): Effect of age groups on appearance of HBsAg positivity among healthy individuals

Age groups/ Years	No. (%) of participants	Mean \pm Std
\leq 20	5 (12.2)	16.3 \pm 1.1
21- 40	25 (60.98)	29.3 \pm 5.5
41- 60	11 (26.83)	47.9 \pm 8.9
Total	41	32.1 \pm 11.8
Statistical analysis	F value= 26.5; DF:2; $p <$ 0.001*	

The asterisk indicates to significant increase with the age progressive ($p <$ 0.001).

Although this study showed that males and females were not significant to the presence of HBV infection, there is the highest significant increase of the viral infection obtained among married (Chi-square= 21.16, $p < 0.001$) females and males. Figure (1) demonstrated that the numbering of HI had HBsAg positive, which comprised 73.3% in married and 26.7% in single during this study.

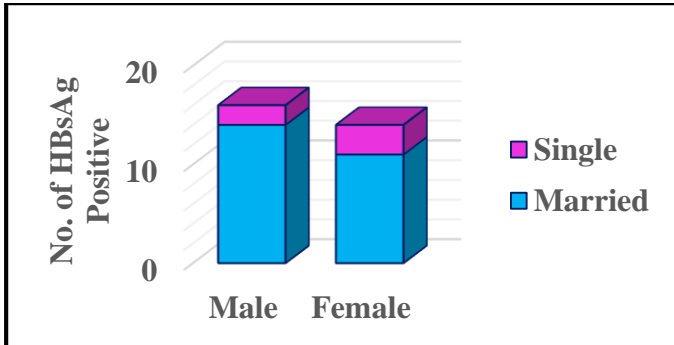


Figure (1): Positive cases of HBsAg among healthy individuals according to sex.

This finding pointed out the reveal of viral infection mostly in individuals aged more than 30 years old accompanied by an increase in sexual contact (Figure 2). The asterisk indicated significant incidence depending on the age of these two attributing influences (Chi-square= 38; $p < 0.001$).

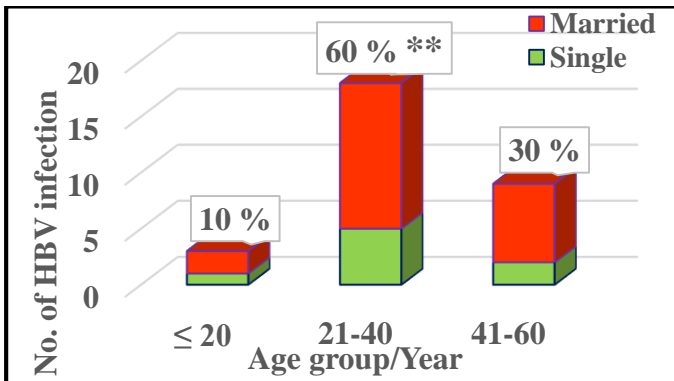


Figure 2: Distribution of HBV infection in different age groups among individuals as sub-clinical cases.

Prevalence of Subclinical IgM-HCV Positivity:

Results showed that 13.6% (34/250) of healthy individuals (HI) had a positive reaction IgM as a sub-clinical case, and there is significant disappearance of the HCV Ag among HI (Chi-square= 132.5, $p < 0.001$). A higher frequency of viral occurrence was reported in ages between 21 to 40 years old, and a significant increase in males 64.7% than in females 35.3% (Chi-square= 9, $p < 0.01$). Moreover, a significant difference was observed among study age groups as summarized in table (2).

Table (2): Effect of age groups on the appearance of HCV-IgM positivity among healthy individuals

Age groups/ Years	No. (%) of participants	Mean ± Std
≤ 20	4 (11.8)	12.8 ± 1.5
21- 40	20 (58.8)	27.4 ± 4.9
41- > 60	10 (29.4)	55.1 ± 8.3
Total	34 (100)	33 ± 15.3
Statistical analysis	F value= 101.5; DF:2; $p < 0.001$ *	

The asterisk indicates to significant increase with the age progressive ($p < 0.001$).

Furthermore, this study showed that HCV infection significant appearance in males than females, there is a higher significant increase of the viral infection obtained among married (Chi-square= 33.64, $p < 0.001$) females and males. Figure (3) demonstrated that the numbering of HI had HCV-IgM positivity, which comprised 79.4% in married and 20.6% in single during this study.

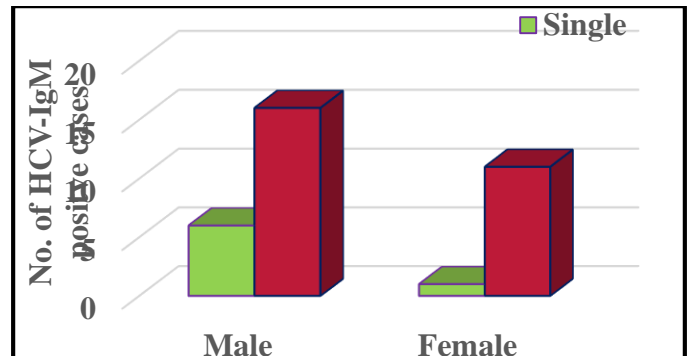


Figure 3: Positive cases of HCV infection among healthy individuals according to sex.

This finding pointed out that viral infection mostly in individuals aged more than 30 years old was accompanied by an increase in sexual contact (Figure 4). The asterisk indicated significant incidence depending on the age of these two attributing influences (Chi-square= 34.1; $p < 0.001$).

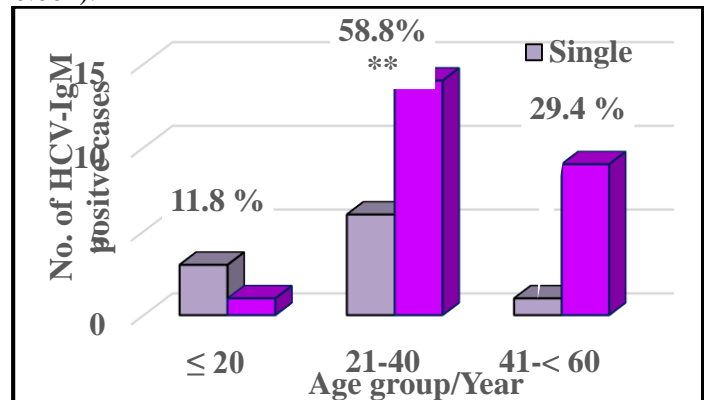


Figure (4): Distribution of HCV infection in different age groups among individuals as sub-clinical cases.

DISCUSSION

Owing to this significant gap between medical knowledge and practices, it has become evident that the graves of patients suffering from various diseases are unfortunately being overlooked and unrecognized. Such negligence is particularly prevalent in rural communities, where subclinical cases often go undetected, gradually progressing into chronic hepatitis. Surprisingly, the presence of subclinical cases in these patients has had minimal impact on the functioning of the liver, as observed in this comprehensive cohort study, marking the first report on the serologically positive subclinical incidence of HBsAg⁽¹⁰⁾.

Hepatitis viruses continue to pose a substantial threat to global liver health, significantly contributing to the overall burden of liver disease. The prevalence of HBsAg and anti-HCV positivity has been extensively studied in various research endeavors, with Turkey presenting figures ranging from 4% to 40% and 2% to 46%, respectively. However, in light of current guidelines and recommendations, the HBsAg test has emerged as the gold standard for screening HBV infection⁽¹¹⁾. Interestingly, individuals with a positive HBsAg result displayed elevated levels of liver enzymes, specifically ALT and AST. This finding strengthens the correlation between HBV infection and hepatocellular damage, highlighting the urgent need for effective interventions and preventive measures. Currently, hepatocellular carcinoma (HCC) ranks among the top five most common malignancies, emphasizing the severity and far-reaching consequences of hepatitis B⁽¹²⁾. In a separate study conducted by these same authors, a normal subject with HBsAg positivity and HBV DNA negativity was examined, revealing significant histopathological injury to the liver. Notably, the subject's liver biopsy showcased the presence of ground glass cells, an alarming indicator of liver cell alterations resulting from the accumulation of HBs antigen. Moreover, the integrated HBV DNA was detected within the hepatic tissues, further reinforcing the correlation between the presence of HBV and hepatocellular changes^(13, 14). While, it may seem improbable, the identification of occult hepatitis B in these subjects can be achieved through traditional (or linear) PCR techniques commonly employed for investigation purposes⁽¹⁵⁾.

Alternatively, it is advisable to consider the utilization of a novel and sensitive HBV-DNA molecular investigation method, such as PCR hybridization, to maximize the accuracy and specificity of the diagnosis process. By embracing these advanced techniques, healthcare professionals can gain deeper insights into the occult nature of hepatitis B and contribute to enhancing the overall management and treatment strategies for this complex condition⁽¹⁶⁾.

Hepatitis B and C are leading infections found in human populations globally. Both viruses can cause acute and chronic hepatitis, as well as a long-term carrier state. In this carrier state, the presence of HBsAg (Hepatitis B virus surface antigen) and HCV core antigens can be persistently detected in the serum. The serological analysis of HBV condition is carried out by investigating HBsAg and anti-HBc (Hepatitis B core antigen), while anti-HBc serves as an indicator of occult HBV infection⁽¹⁷⁾. It is important to note that certain cases of occult hepatitis appear serologically healthy and test negative for HBsAg. These subclinical cases have been documented in studies conducted among healthy subjects globally, which have also reported the prevalence of HBV, HCV, and their co-infections⁽¹⁸⁾.

Given the significance of this global problem, it is crucial to conduct comprehensive investigations in various countries. These investigations will provide a better understanding and evaluation of the real condition of both infections, considering the rural and urban populations within each area. By expanding research efforts to cover a wide range of populations, we can enhance our knowledge and develop effective strategies for prevention and treatment⁽¹⁹⁾.

The importance of early detection of these diseases is critical to control the horizontal and vertical transmission of these viruses and eradication of them from a population⁽²⁰⁾. WHO has declared an ambitious target of eliminating both these diseases by 2030, however, this ambitious target could not be achieved without early detection of these diseases and subsequently the eradication of viruses from the population. The mode of transmission of HBsAg and IgM-HCV is parenteral. The likelihood of being exposed to these viruses increased in minor surgical procedures in rural areas, blood transfusion, and sharing of syringes. Similarly, dental procedures without sterilized instruments are a common mode of transmission. Unsterilized needles used in quack practice, barbers, and nonsurgical cosmetic procedures are other common modes. Due to these reasons, these high-risk groups are relatively more likely to have these diseases^(21, 22).

Other factors that increase the risk of viral infection include poor hygienic practices, a history of blood transfusions, multiple surgeries, use of injections, and dental surgery⁽²³⁾. The prevalence of HBV and HCV was observed more in men and commonly those of middle age⁽²⁴⁾. HBV is known to be mainly transmitted by parenteral, sexual, and perinatal routes; mother-to-child transmission at birth and horizontal transmission is very frequent during early childhood in 80 to 90% of HBV-infected newborns in the absence of any prevention⁽¹⁸⁾. HBV is relatively resistant to environmental stresses and can survive outside the body for a week or more on dry surfaces, which can lead to viral transmission through

open wounds, skin infections, mucous membranes, etc⁽²⁵⁾. Approximately over 95% of HCV infections are sexually transmitted, not parenterally. However, prompt appropriate knowledge should be kept in view to avoid susceptibility to HCV infection through factors: drug addiction, minor and major surgeries, barbers' shops and hemodialysis, etc. The major causes of viral spread are needle sharing, open wounds, and razor or nail cutter sharing^(26, 27).

CONCLUSION

In conclusion, these key findings emphasize the urgent need for improved healthcare strategies aimed at preventing and managing HBV infections among Iraqi healthy individuals. This includes increasing access to vaccination programs, enhancing screening processes, implementing effective treatment options, and raising public awareness about the risks associated with HBV infection. Also, understanding the prevalence of IgM-HCV-positive cases among healthy individuals can assist public health efforts in identifying and targeting populations at higher risk for hepatitis C infection.

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