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Evaluation of Serological and Molecular Detection Methods for HBV and its Associated Risk Factors among General Population in the Province of Babil/ Iraq

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ABSTRACT

In Babylon Governorate, the frequency of viral hepatitis B in the general population was evaluated. A total of 354 members of the general community with ages ranging from <15 to >45 and a mean of 38.64 years old were randomly selected for the study and tested for anti-HBsAg, HBc IgM and Viral load. The findings of the current study showed a prevalence rate of 3.95% of HBV in the general population where fourteen samples had positive HBsAg while the highest risk of infection (5.6%) was found for individuals older than 45Yrs. According to gender, males were more likely to be infected than females (10:4 vs. (71:4:28.6), respectively. For the residency status, the rate of infection was 2:1 for the total of (14) positive anti-HBsAg, of which nine were in rural areas and five of them were in urban areas. Compared to anti-HBsAg, which was obtained by an ELISA test, the diagnostic marker anti-HBc IgM was more consistent with the viral load/Ml of HBV determined by RT-PCR.

KEYWORDS: HBsAg , HBc-IgM, ELISA, Minividas, RT-PCR

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INTRODUCTION

The Latin word hepatitis refers to hepatic inflammation (Mehwish et al., 2011). Acute hepatitis is a brief illness that resolves fast, but chronic hepatitis results in long-term illness. The severity of hepatitis relies on a number of variables, including the cause of the liver injury and any underlying disorders in the body (Jou and Muir, 2008). In certain cases, it may result in liver damage, liver failure, or even liver cancer. About thousands of individuals each year pass away from the effects of hepatitis B, which has infected two billion people globally (WHO, 2012). Anti-HBsAg prevalence differs between the region's nations: 4%–5% in Iraq, 3%–11% in Egypt, 2.6%–10% in Jordan, 2%–6% in the Libyan Arab Jamahiriya, 2.3%–10% in Oman, 5%–6% in Palestine,7.4%–17%in Saudi Arabia, 16%–20% in Sudan,6.5% in Tunisia, 2%–5% in United Arab Emirates and 12.7%–18.5% in Yemen (Wasfi and Sadek,2011).

HBV is a compact genome with 3200 nucleotides long and possesses a circular shape of partly double-stranded DNA (Thomas et al., 2005). The entire HBV virion with infectivity can be seen under electron microscopy as a 42–45 nm long spherical form known as a "Dane particle" (Lee and Ahn, 2011). The shells of the virus are composed of two layers. The outer shell is composed of the envelope protein known as HBs protein, which is further subdivided into small, middle, and large HBs proteins (SHBs, MHBs, and LHBs proteins, respectively). The inner shell is composed of a core protein known as the HBc protein, which encloses viral polymerase as well as the HBV genome (Ganem and Prince,2004). The current study's objectives are; Assessment of various HBV detection methods, including ELISA, Minividas, and real-time PCR. Examine the prevalence of HBV in the province of Babylon.

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MATERIALS AND METHODS

Distribution of the samples:

The study population comprised total samples of 354 of randomly selected individuals, and were recruited from the central public health laboratory, blood bank, Hilla teaching hospital, and Marjan teaching hospital in Babylon province. Their age ranged from <15 to >45 years, with mean age of 38.64 years.

Written informed consent was obtained from all of the participants and/or their parents. Designed questionnaires were administered to obtain demographic data from the study group. Negative control were those apparently healthy blood donors who are not suffering from any acute ailment at the time of sampling along with a proper vaccination record. Positive control were those suffering from liver diseases and underlying similar health conditions who have improper vaccination records.

Blood collection:

Each participant's venous blood was drawn in the public health laboratory. For plasma collection, one ml of anticoagulant (EDTA) was combined. The remaining samples were used to collect serum, which is spun at 1000 rpm for 5–10 minutes to separate it, and then it is chilled or frozen at -20 until it is utilized for the appropriate test. Acon-USA's third-generation enzyme immunoassay kit (EIA-3) was used to determine anti-HBsAg levels. The automated VIDAS system's enzyme-linked fluorescence immunoassay (ELFA) (Minividas Kit/HbsAg, Biomerieux-France) was used to confirm the anti-HBsAg reactivity. The viral load was then determined by RT-PCR utilizing the Exi-prepTM viral DNA/RNA kit (Bioneer-Koria) to extract the virus DNA and the RT-PCR Amplification kit (Sacace-Italia) to amplify the viral DNA.

RESULTS AND DISCUSSION

HBV infections start when the immune response that usually clears the virus does not function properly or is insufficiently strong to be effective; as a result, infections are more prevalent among people with low immunity due to poverty (1997, Hoofnagle). As described in table 1, the current study found that the prevalence of anti-HBsAg in the general population was (3.95%). For the age group >45 Yrs, having a greater prevalence of infection (5.6%) than the age group (25–44 Yrs), which had a prevalence of (5.5%). This may support the idea that these age groups are more exposed to the risk factor of infection. This result might be attributed to the exposure of live activities including; sexual activity, work, or travel. Statistical analysis showed a correlation between rate of infection and the age of infected individuals in these age groups (p 0.05).

Table (1): Frequency distribution of anti-HBsAg in general population by ELISA test

Age groups	No of samples tested	Anti-HBs Ag+	Anti-HBc IgM+		ELISA		
			NO	Index	Mean of the O.D (650nm) of negative control	Mean of the O.D (650nm) of positive control	Mean of the O.D(650nm) of patient samples
<15	95	2	1	0.67	0.011	0.496	1.64
15-24	98	4	3	0.89	0.034	0.360	1.42
25-44	72	3	2	0.83	0.03	0.322	1.86
>45	89	5	1	0.49	0.06	0.400	2.00

The statistical analysis relieved significant difference between the mean of optical density of patients samples and negative control for all age groups ($LSD_{(0.05)}$1.783).

Figure (1): Frequency distribution of anti-HBs-Ag in different age groups of general population by ELISA test.

Table 1 showed that the prevalence of anti-HBsAg in the general population was (3.95%), and that out of a total of 14 positive samples, 7 also tested positive for anti-HBc-IgM, a marker for recent or acute HBV infection (Yin and Tong, 2006) . This finding was also supported by (Al-Awady et al., 2008), who explained that the infection might occur primarily at birth due to vertical transmission (from mother to child), the age of presentation is between 25 and 35 years old because the patients are asymptomatic and were unintentionally identified by routine testing while giving blood, getting married, or working, all of which are common activities for people in this age range. According to several risk exposures of ageing groups, such as injections with syringes, blood transfusions, and invasive operations, the incidence of infection in the older age groups can be therefore explained. Numerous findings published by Allwright et al. (2000), Sandesh et al. (2006), and Memon et al. (2010) supported the findings of the current investigation. According to gender; male was more prone to infection with HBV than female (10:4) with the rates of (71.4: 28.6) as shown in figure (2):

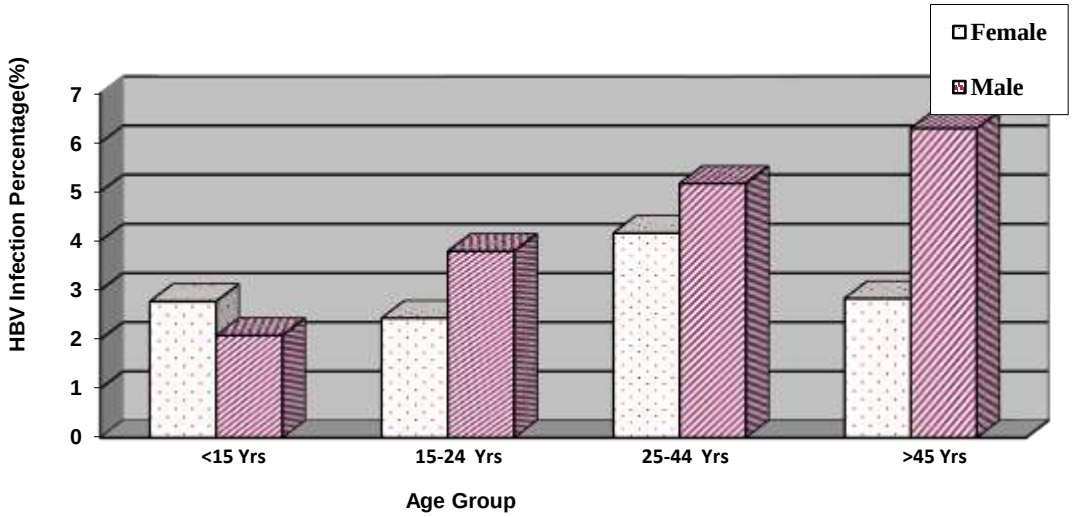

Figure (2): Frequency distribution of HBV infection according to age and gender among general population.

The outcome of the current study is in consistent with other studies, such as Khan's (2011). Additionally, (Dennis et al.,2005) and (Alexander et al.,2007) found that men predominate over women in all populations of anti-HBsAg carriers, supporting the well-documented fact that higher anti-HBsAg seroprevalence has been reported in male than in female for populations in some Asian countries. Women are more likely than men to clear anti-HBsAg, despite the fact that this is a well-established but poorly understood factor in chronicity (David and Daniel, 2003). These findings support earlier research in this area conducted in Iraq (Husain, 1997) and 2012 (Heim). There were a total of 14 positive anti-HBsAg tests; 8 of them were in urban areas, and the remaining 6 were in rural areas, with a rate of infection of 2:1, as illustrated in Table (2). In this study, the hepatitis B virus distributions by place of residency showed that there was a significantly higher prevalence of the virus in urban than rural areas ($p > 0.05$). This conclusion could be the result of improved health education in urban than rural regions, which promotes early disease diagnosis. The increased frequency of HBV in urban regions may be attributed to the crowded nature of cities, which may promote HBV and HCV transmission. These findings are consistent with those of earlier research conducted in Iraq by Hussin (1997) and Al-Awady (2008) . The distributions of the hepatitis B virus in this study showed a significant difference ($p > 0.05$) between the prevalence of the virus and the economic status; with high prevalence of the virus in population living with low economic status, compared to medium economic status and the lowest prevalence was found for those living with good economic condition. For HBV, the proportion of those with good, medium, and low economic standing was 1:1:2, respectively. The current study supports earlier research by Mistik and Balik (2001), which revealed that those with lower socioeconomic status and less hygienic living conditions are more likely to contract HBV than other people.

The result of the current study is consistent with the findings of other studies conducted in Japan (Dennis et al., 2005), which demonstrated that lower socioeconomic states have higher rates of HBV prevalence. These results were confirmed by additional findings published by Alter (1993) and Murphy et al. (1994). The distributions of the hepatitis B virus in this study, according to the educational level, showed that patients with low educational level (primary and secondary school education) compared to those with high educational level (graduate and post graduate education), showed a high prevalence of HBV with a significant difference ($p >0.05$). According to this study's findings, individuals with lower educational levels are more adversely

affected by HBV at a ratio of (6:1) than those with higher educational levels.

Table (2): Frequency distribution of infection ratio with HBV according to residency, economic status and educational level.

Associated parameter	Hepatitis B Virus		
	Frequency		Infection ratio
Residency	Urban	125	(8\125)= 6.4
	Rural	229	(6\299=2.6)
Economic Status	Good	59	(2\59=3)
	Medium	118	(6\118=5)
	Low	177	(6\177=3)
Educational level	High	118	(2\118=1.69)
	Low	236	(12\236= 5.1)

Table (3): Frequency distribution of anti-HBsAg for different age groups by ELISA, Minividas and RT-PCR in general population.

Age groups	Anti-HBs Ag+(ELISA)		Anti-HBs Minividas value IU/Ml		RT-PCR	
	No of samples tested +ve.	Index	+ve	Value	+ve	Viral load IU/Ml
<15	2	20.5	2	15.14	2	$13.07*10^7$
15-24	4	14.2	3	13.90	3	$85.4*10^5$
25-44	3	20.0	8	17.80	8	$11.06*10^6$
>45	5	15.72	13	17.52	13	$1.668*10^7$

Although the higher mean titers for both ELISA and RT-PCR correlated (2.00 and 1.668*107, respectively), As illustrated in table (3), the index mean of anti-HBsAg produced by ELISA technique does not entirely correlate with viral load of the virus. As shown in table (3), this was also attained at a lower mean titer. This might be attributed to to the fact that all results were obtained automatically by the apparatus which measured the results value depending on Calibrator 1 (represent positive control) and Calibrator 2 (represent negative control). The anti-HBsAg prevalence was also examined by Minividas technique and were more specific compared to ELISA technique. By using the cutting-edge molecular technology RT-PCR, the viral load of the positive specimens was also assessed. This study shows that manual and automated procedures were consistent across all examined blood samples. Since the employed kit of (IgM-HBc) was based on the competitive combination principle, the results in Fig. 3 in the general population group refer to positive correlations with (IgM immunoglobulin) as a component of adaptive immune response and viral load of RT-PCR test. These findings are consistent with a previous study from Han et al. in 2008, who discovered that the IgM anti-HBc and HBV DNA viral load combination has a positive coloring and enhanced diagnostic capability. The high viral load titer in children under 15 who were infected can be attributed to the fact that their mother was either unvaccinated or diseased when they were born, or it could be due to a contaminated device or a hospital-related infection (nasocomial infection). Since the majority of those individuals in this age group were +ve for anti-HBc IgM, the higher viremia observed in the age group (31-40) indicates that there are newly infected individuals in this age group. Higher age virus loads exhibit the gradual reduction. This may be due to the fact that these groups were exposed to the infectious agent before the diagnosis was made and that because of their advanced age, they were more likely to undergo medical procedures and receive blood transfusions.

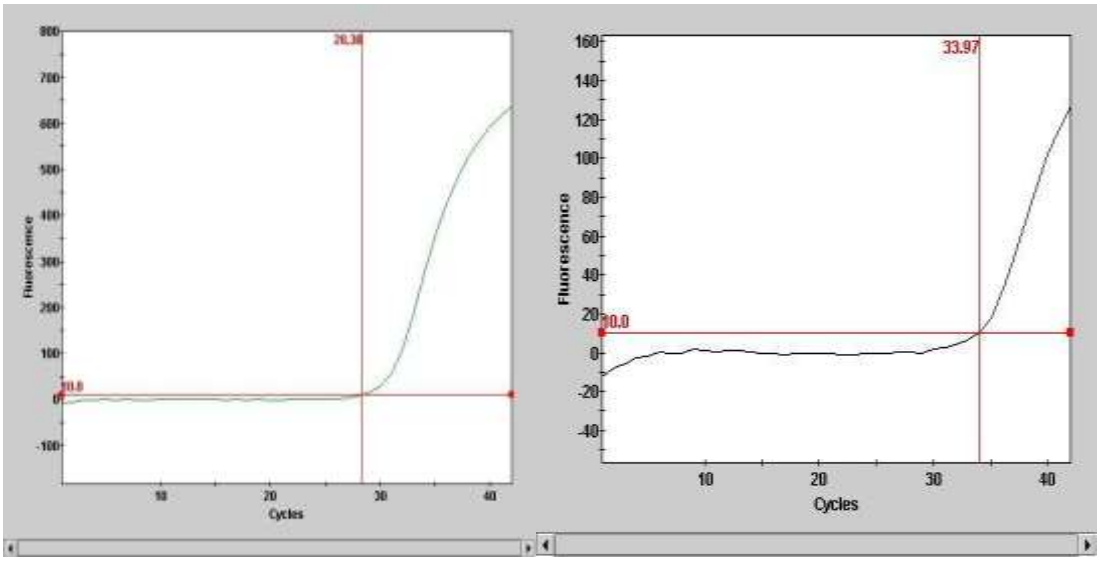

Figure (3): Relative fluorescence vs – cycle number – amplification plot showing two positive cases with two different (Ct)s (28.3 and 33.9 which indicates different levels of viral genome.

CONCLUSION

In Babylon province, the prevalence of HBsAg among the general population is 3.96%, which is significantly greater than the prevalence of HBsAg among healthy blood donors (0.76%). Significant correlations were found between the HBsAg infection and male sex, urban areas, low socioeconomic status, and low educational attainment. Hepatitis B disease prevalence can be evaluated by determining the viral load of the infection using RT-PCR.

Compared to anti-HBsAg (ELISA), combining the diagnostic marker anti-HBc IgM with the viral load/Ml of HBV (RT-PCR) showed consistent results and improved test capability, therefore combining the latter diagnostic tests is recommended.

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