

Serological Study of Hepatitis B Occult Among Blood Donors and Couples in Al- Najaf Province

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Abstract

Transfusion is a major problem for viral hepatitis even after the HBsAg test is approved by Rapid Test. Note that it is the only test used by some blood banks and some centers for those who are about to get married. However, HBsAg-negative individuals do not exclude the risk of transmission of hepatitis B, as it may be in the 'window period' or have a mutant strain. Detection of antibodies during this period to HBcIgM represents a useful and more accurate serological marker. This study aimed to evaluate the importance of anti-HBc IgM screening of HBsAg-negative blood donors and future married couples in order to reduce the risk of hepatitis B virus transmission in Al-Najaf Governorate. The current study was conducted in the College of Education for Girls, University of Kufa, Department of Life Sciences. Samples were collected on 10/21/2020 until 1/2/2021 from the Central Blood Bank and the General Hospital in Al-Manathrah in Najaf Governorate. 546 serum samples were collected from two sources: 450 male blood donors in the main blood bank and 96 newlyweds - of both sexes - (55 females and 41 males). Rapid serological tests were applied for the detection of anti-HCV, HIV1/2, HbsAg, ELISA HBcAg test and Nested PCR test, which were conducted in the laboratory of Iraqi biotechnology companies in Al-Diwaniyah Governorate. The results showed that all samples examined by Rapid Test were negative for HBsAg. When conducting the ELISA test, it was detected that 30 samples were 5.49% positive for HBcAg, and the results of the study showed that there were no significant differences between the source of the sample for blood donors and those who are about to marry, although the infection rate of males was higher than females, and there were no significant differences between the sexes at the probability level of 5%. With regard to ages, it was found that the highest rate of infection was within the older age groups 58-68 years 23.52% compared to other age groups. There are no significant differences at the 5% probability level for the nature of housing.

1. Introduction

hepatitis virus HBV It is one of the pathogens spread all over the world] WHO.,2017. [More than 2 billion people are infected HBV Worldwide in ,2017 the 257 million people infected with hepatitis B People with hepatitis B Chronic ,which is associated with serious complications including cirrhosis and hepatocellular carcinoma ,with an estimated 887,000 deaths annually. {WHO.,2019} The risk of transmission of hepatitis virus is associated with B Mainly with negative blood donations for HBsAg that have been collected during (window period) Pre-seroconversion which is defined as the time between infection and detection of the surface antigen of the virus ,or cryptic transmission of hepatitis infection B (OBI) Candotti and Allain. , (2009) .has been defined OBI as having HBV DNA without HBsAg discoverable outside the window period .It is a special form of hepatitis virus infection B And a recurring phenomenon that was first reported in a year1978It has been better studied than then Hoofnagle et al., (2007) ,and was known by experts in general 2008 It is the presence of DNA with the amount HbsAg In the liver of individuals negative for surface antigen tests HbsAg or existence

HBVDNA in serum less than 200IU/ml or undetectable Raimondo et al., (2008a) It is a global problem all over the world. carry OBI Potential risks of transmission HBV Through transfusion of blood and its derivatives, through sexual contact of all kinds, from the infected mother to her fetus (vertical) in the perinatal period .The perinatal period is the main mode of transmission of the hepatitis virus B at the level of the world . Tran., (2009)

The Study Groups

The patients were classified into the following groups according to: Ages of Patients:

A1	Patients aged from (14-24)
A2	Patients aged from (25-35)
A3	Patients aged from (36-46)
A4	Patients aged from (47-57)
A5	Patients aged from (58-68)

Sample type

1. (S1) Patients with viral hepatitis pattern - me on the verge of marriage.
2. (S2) Patients with viral hepatitis pattern - my donor blood.

Gender type

1. male (M) The number of males infected with viral hepatitis pattern B
2. female (F) The number of females infected with viral hepatitis pattern- B

2. Residency

1. countryside Rural (R)
2. city Urban (U)

Study Design

The current study included

1. Total number of people under study= 546 Person
2. number of patients= 30 Person.

Method of work

The study involved 546 people who were divided into two groups. The first group included 96 people from prospective marriage (55 females, 41 males) and 450 blood donors - all male - who tested negative. HBsAg by Rapid Test was randomly selected from the General Hospital in Manathera and the main blood bank in the county, the

diagnostic kit was used to test HBcIgM by ELISA Bio Test and the test was done according to the manufacturer's instructions, then the positive samples were tested for anti-HBcIgM for HBV DNA by PCR.

3. Results

Demographic information for the study

This study was conducted on 546 serum samples from blood donors and healthy couples. All samples were subjected to a rapid blood test by Rapid Test to detect the possibility of the presence of antibodies to each virus) HBsAg \HCV\HIV (The results showed rapid test that all samples under study negative for blood tests The results of the statistical analysis showed that there were significant differences at the probability level of) %5 Table(1-4) .

Table (1-4) of the frequency distribution of study groups according to social and demographic variables.

Variable	category	The number	Percentage	X2	P value
Sample type	Blood donor	450	82.417	459.03	<0.0001
	Couples	96	17.582		
	The total number	546	100		
Gender	male	491	89.926	696.3	<0.0001
	Female	55	10.074		
	The total number	546	100		
Age categories	14-24	95	17.399	215.10	<0.0001
	25-35	172	31.501		
	36-46	182	33.333		
	47-57	80	14.652		
	58-68	17	3.113		
	The total number	546	100		
Residency	Urban	386	70.695	187.09	<0.0001
	Rural	160	29.304		
	The total number	546	100		

Frequent distribution of study groups according to the source of the sample:

Table (4-1) indicates the division of samples according to the source of the sample into two groups. The first group included blood donors who visited the main blood bank during the study period, where the total number was 450, at a rate of (82.417%), while the sample group was from the next on marriage for the same period, their number is 96, or (17.582%) .

Frequent distribution of study groups by gender

Table (4-1) shows the frequency distribution of study groups by gender, where the percentage of males who visit the main blood bank for the purpose of donation and those who are about to get married in the General Hospital in Al-Manathera is the highest with a percentage of (89.926%), while the percentage of females is (%10,074)

Frequent distribution of study groups by age

Table (4-1) shows the frequency distribution of the

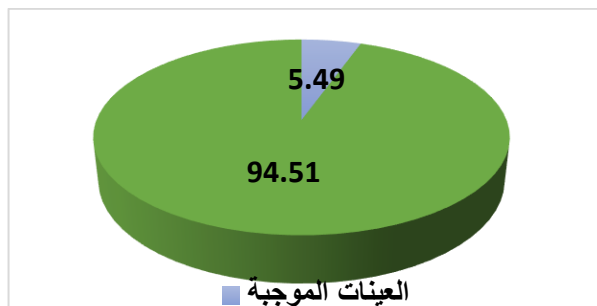
study groups by age, where the statistical indications showed that the age group (36-46) years was the largest number among the study samples with a percentage of (33.33%), followed by the age group (25- 35) years with a percentage of (31.501%), while the age group (58-68) was the least in number among the groups, at a rate of.(%3.113)

Frequent distribution of study groups by area of residence

Statistical study also indicated that the reviewers who live in the city in the samples under study group is the highest percentage (70.695%) compared to those who live in rural reviewers where the proportion of (29.304 regions%) of the study sample group as Table (1- 4)

4. Test Results of ELISA

Test results showed ELISA of the 546 samples that were negative for surface antigen HBs A g by Rapid Test 30 antigen-positive samples were detected HBcAg by 5.49% Of the total samples under study form(5 - 4) .



shape (5 - 4) test results for the immune antigen HBcAg in patients with surface antigen negative HBs Ag using test ELISA.

Distribution of patients with viral hepatitis pattern– B occult according to the source of the sample:

The surface antigen-negative and antigen-positive samples were divided HBcAg According to the source of the sample, it was divided into two groups : the first group included 450 blood donors , all of them were males, the number of infected people was ,27 at a rate of ,(6%) while the second group included 96 people between male and female marriage contracts, the results showed that three people were infected with a percentage of (%3.12) with no There are significant differences at the %5 probability level 1 Table(4-2).

Percentage	Positive number	The number	Sample type
6	27	450	Blood donor
3.12	3	96	Couples
5.49	30	546	The total number
* 1.25			X2
0.262			P value
*There are no significant differences at the level of probability5%			

Distribution of patients with viral hepatitis pattern– B occult by gender:

The antigen-positive samples were divided HBcAg Depending on gender into two groups Male ,Female, The results showed that the highest rate of infection of hepatitis viral pattern-B The male group had 28 infected (5.70%), while the number of infected females was 2 with a percentage of (3.63%) with no significant differences at the 5% probability level Table (3-4).

Percentage	Positive number	The number	Gender
5.70	28	491	Male
3.63	2	55	Female
5.49	30	30	The total number
* 0.407			X2
0.524			P value

Distribution of occult viral hepatitis patients’ pattern – B by age:

The antigen-positive samples were distributed HBcAg According to the different age

groups into five groups ,the first age group from 14 to 24 years, the total number of this group was 95 people, and it included one patient with a rate of (1.05%), the second age group included ages from 25 to 35 years, the total number of this category was 172 people, and the number of infected people reached 10 with a rate of (5.81%) The third age group included ages from 36 to 46 years, the total number of this group is 182 It included 7 patients with a percentage of (3.84%), the fourth group included ages from 47 to 57 years, the total number of this group was 80 people, 8 patients were infected with a rate of (10%), and the last group included ages from (58 - 68 years) the total number of these The group of 17 people included 4 patients, whose percentage was (23.52%), which is the most affected group, while the results showed that the least affected age group was **24-14** years with a percentage of (1.05%), with significant statistical differences at the probability level of 5% Table (4- 4).

Percentage	Positive number	The number	Age categories
1.05	1	95	14-24
5.81	10	172	25-35
3.84	7	182	36-46
10	8	80	47-57
23.52	4	17	58-68
5.49	30	546	The total number
* 18.37			X2
0.001			P value
*There are significant differences at the level of probability5%			

Distribution of patients with viral hepatitis pattern– B by residence:

Samples of antigen-positive patients were distributed HBcAg by residence to city and countryside, the highest percentage of infection among the group of patients living in the city was (6.21%) compared to the group of patients living in rural areas at a rate of (3.75%), with no significant differences at the 5% probability level Table (4-5).

Percentage	Positive number	The number	Residency
6.21	24	386	Urban
3.75	6	260	Rural
5.49	30	546	The total number
* 1.32			X2
0.249			P value

5. Discussion

Detection of surface antigen using rapid tests is Rapid Test Inaccurate in test results ELISA Figure (4-1)The current study showed the inaccuracy of the results of the rapid examination of samples tests in detecting hidden infection with hepatitis type-B .The reason for this is due to the presence of very

low concentrations of the virus in the blood (Viremia) It cannot be detected, since the surface antigen has taken place during the period window Period During which it is not possible to determine the surface antigen of the virus and which lasts (6-10) weeks Al- Azi .,(2012) ,Is the use of a number of different laboratory of origin give different degrees sensitivity , which affects the results of the study or the occurrence of mutations in a genes or pre-S It may be due to a gene rearrangement S Which leads to protein synthesis .Sate antigenically can't be detected by tests HBsAg Commercially available, therefore, screening for hepatitis B virus pulp antibodies using a technique ELISA Among the confirmatory tests for blood donors as well as the polymerase chain reaction test PCR Screening for HBV DNA for negative cases of the surface antigen screening test HBSAg .Prepare anti- HBc IgM the first specific antibody to appear after exposure to acute infection category IgM of antigens HBc Even late in the incubation period and indicates a recent infection .So, the anti HBc IgM Is the sign of infection HBV in negative blood donors HBsAg .while showing a category IgG of antigens HBc Later, it indicates a previous injury .Individuals with antigens may not be HBcIgG They are infectious and their blood is suitable for transfusion as they may have sufficiently high titers of antigens HBs of a protective nature Al- Azi ., (2012) Even during the window period, its concentration decreases after 6 months and may be determined again during the recovery of the injury Beranger and wright , (2002). The results of our current study showed that out of 546 samples negative for surface antigen HBsAg There were 30 samples positive for pulp antigens HBcAg by (5.49%) of the total samples under study using an assay ELISA and 29 samples (%96.66)were positive for .HBV DNA using a device Nested PCR Although they are negative for the surface antigen screening test HBsAg for blood donors and those who are about to marry who do not suffer from diseases .The results of the current study were in agreement with a study conducted in Iran Pourazar *et al.*, (2005) Out of the 545 samples collected from the blood transfusion organization in Isfahan, 43 samples were positive for antibody HBc and negative for HBsAg the data showed that 545/5 samples were positive for .HBV DNA and that the donors of these samples have a hidden infection .It is compatible with a study conducted in Egypt by the researcher Marwa *et al.*, (2020) Conducted on 400 plasma samples from healthy blood donors who were negative for all blood bank tests,) HBV, HCV HBsAg ,HIV, Spilla (There were 9/400 positive for. antigens HBc and by 5.25%, seven of them were males and two were females, and 4 out of 9 samples with a percentage of 1% were positive for HBV DNA by device PCR .The study showed a relative

difference from other studies conducted by the researcher) Shiha .,(2011 In Palestine, out of 489 blood donors, the negative for HBsAg There were 49 positive antigens HBc with a rate of 10.96%, and another study was conducted in Egypt with a rate of 10.96%) .,(2008 El- Zayadi The percentage in the results of a study conducted in the Kingdom of Saudi Arabia was 17.4%) .,(2001 Ankra –Badu While the results were low among Lebanese blood donors by) %3.7 Ramia *et al.*, (2005 .) On the other hand, the results of the current study were higher than those recorded before Essam., (2007) In Egypt, which included 712 samples that were routinely examined by blood unit tests (HIV, HCV, and syphilis) Where the results of that study were 0.13%-unit positive for antigens HBc -IgM and 0.15 % positive unit of HBV DNA. Another study was conducted in India by Lavanya .,(2012) It included 12232 negative samples for HbsAg The results of this study showed that 0.12% units are positive for antigen sHBc -IgM It is a low percentage compared to other studies due to the increase in the number of people in the study.

A group of Egyptian blood donors, 1026 blood donors, all tested negative for HBsAg to find out the presence of antihistamines HBc and reported that it was found 80/1026 (8.7%) blood sample for anti for anti - HBc IGg and it was discovered HBV- DNA in a)5. (%4of these samples, (2008) El- Zayadi .as he did Tamer and his colleagues, in Gharbia Governorate, Tanta University, conducted two recent studies of a high-risk group for transmission HBV .Where the first study included 90 samples of regular hemodialysis patients who were negative for the virus HBs Ag and anti HCV .has been tested PCR HBV- DNA The results included 17/90 with a positive rate of 18.9% for . HBc IgM Seven of them were positive HBV-DNA In 7.8% of all patients Abdelrazik ., (2015) The second study included 79 negative urinary tract infections HBsAg ,HBsAb and anti HBC Ab Patient samples were tested for total antibody HBC Out of 79 negative serums HBsAg 79/12positive units for . were detected HBcAg and by 15.19% of all patients 5/5 .positive units detected HBV-DNA and by 6.3 %of all patients) Elbedewy. , (2016 .) Japhet *et al.* ,(2011 Found a spread for anti- HBc IgM By only 5.4% of blood donors Moajabin but did not look for anti-depressants HBc the college . is found Salawu *et al.*, (2011) Also, about 4.4% of the samples were positive for antigens HBC and negative for HBsAg the rapid test kit was used in this study and is less sensitive than ELISA used in this study.

The results of our current study, as shown in Table (4-2) Figure (4-1), showed that there were no significant differences between the source of samples with respect to the number of positive samples compared to the total number of samples for both types of samples, meaning that there is no significant effect of the source or the type of sample on the incidence

of hepatitis virus pattern - B invisibility.

The results of our current study, as shown in Table (4-3) Figure (4-2), showed an increase in males' infection compared to females, although the statistical results indicate that there are no significant differences between the sexes. The reason may be due to the physiological differences between the sexes. The large number of male blood donors, especially in the study area, the efficiency of the immune system in females compared to males, as they secrete estrogen in a greater amount than males, which works to inhibit the action of the enzyme Caspase-12 Which in turn activates programmed death Apoptosis as for the immune cells, and thus estrogen works to prevent the killing of immune cells, so females are more resistant to viral, bacterial, fungal and parasitic infections. Our study agreed with the results of another study reached by researchers Jacobsen and Koopman., (2004) Who showed that the incidence of viral hepatitis pattern -B It is higher in males than in females, and its prevalence is also wide in developing countries that suffer from overcrowding and a clear shortage in health and environmental fields, as another study showed by the researcher Lett., (2013) Which explained that most injuries are through blood, bodily fluids and sexual contact, and the increase in males can also be explained by several reasons, the most important of which is the possibility of male exposure to razor blades or frequent mixing with members of society EL- Gilany and El- Fedawy., (2006) while clear Verscheure et al., (2005) The reason why males are infected more than females is due to genetic factors related to males' predisposition to viral infection more than females, according to the genotype of the virus. slightly higher than the female Kacar et al., (2003)

The results of the current study, as shown in Table (4-4) Figure (3-4) showed clear significant differences in the distribution of patients infected with viral hepatitis type- B According to age groups, these results were consistent with studies conducted in Nigeria and India, which consider age as an important factor in the spread of the virus HBV The highest percentage of HIV DNA was recorded in the age group (15-29) years, and the reason is due to the increase in sexual activity during this period Gerlich et al., (2007); Satake et al., 2007 While the current study was inconsistent with a study conducted in Diyala Governorate, which recorded the highest rate of viral DNA infection within the age group (30-39), it may be the result of not taking the vaccine. Al- Azi., (2012) While our current study showed that the highest incidence of infection was within the age group) 58- 68 (and the proportion of) 23.52 (%and the lowest percentage was within the first age group 14 to 24. The reason may be due to the fact that people of young ages who received the vaccine, as it became widely available as part of the comprehensive immunization schedule in recent years, as well as the ability of the immune system to detect the disease and confront it

in age stages. Early treatment gives a greater opportunity for treatment and recovery, and the survival of the infection for a period of time leads to weakening of the individual's immunity and damage to the liver, which leads to cirrhosis and carcinogenesis of hepatocytes and death may occur. The results of the current study, as in Table (4-5), Figure (4-4), indicated that there were no significant differences in the number of injured people living in the city and the countryside, and they are consistent with the study conducted in Diyala Governorate. Al- Azi., (2012) Which recorded the highest rate of viral DNA in urban areas compared to rural areas, which is in agreement with both India and Nigeria, which consider housing an important factor for the spread of infection Mabayoje et al., (2010); Chandra et al., (2009) This is due to the city being the highest population density and the spread of unhealthy habits, especially in crowded areas, and most of the study samples are city residents. The results of the current study agreed with the study Chamberlain., (2004) Which showed that the rate of risk of infection with hepatitis B virus, whether in rural or urban areas, depends mainly on the difference in living conditions and the extent of attention to personal hygiene.

6. Conclusions

1. Conducting a screening test for HBcAg for hepatitis B virus using ELISA technology in the routine examination of blood donors and those who are about to marry to reduce the risk of HBV transmission as well as reduce the consequences and events for those who suffer from immunodeficiency, as well as in other medical procedures such as dialysis and organ transplantation.
2. A follow-up study is necessary to examine the clinical effects of occult hepatitis C in donors who have tested for anti-HBc reaction.

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