Original Article

Serological Patterns of Hepstitis B Virus Among HBsAg Negative Blood Donors in Baghdad, Iraq by Using Architect System

Zainab S. Hassab*	**BSc. MSc**
Amani K. Abd Al-Ruhman**	**BSc, MSc**
Yakub A. Saleh**	**BSc, MSc. PhD**
Auroba, I. Abd Allah**	**BSc, MSc**

Abstract:

Background: About 10-15% of population HBV infection becomes chronic, and 85-90% are resolved. In Iraq, HBs-Ag assay by ELISA is only mandatory test for detection of HBV infection in blood donors. Anti-HBc and anti-HBs were not detectable in their sera.

Objective: To investigate the distribution of serological patterns for HBV who were HBsAg negative.

Material and method: Between July 18, 2011 to December 25, 2011, 10ml of blood samples were collected from National blood Transfusion Center donors, Baghdad, for 25782 individuals (25294 male, 488 female), mean age (20-65)years. HbsAg positive sera excluded (185), and final number of donors included in the study was 25597 HbsAg negative.

Result: The data showed a previous exposure for HBV($p>0.05$) through anti-HBc and anti-HBs 3.8% by Architect system. The anti-HBs combine with anti-HBc was 68.5%, while anti-HBc alone was 31.5%. Independent risk factors were association with married, history of blood transfusion and residence.

Conclusion: The prevalence of the HBV in Iraq is 4.6% as intermediate region at first time.

Key Words: Serological patterns, HBsAg negative,Architect system,Blood donors.

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Introduction:

Hepatitis B virus (HBV) remain a major health problem(1). Approximately 2 billion people in the world have serological evidence of past hepatitis B virus infection(HBV) and more than 400 million are chronic carriers of HBV(2). Approximately 2 people die each minute due to HBV infection; representing the 10th leading cause of death worldwide(3). HBV is belong to the Hepadnaviridae family(4).

The prevalence of hepatitis B infection has been investigated are: Patient with liver disease, Intravenous drug, addicts, hemodialysis, hemophiliacs, HIV, diabetes mellitus and apparently healthy individuals(blood donors, general population) (5,6,7) HBV is easly transmitted by blood product as a cellular and plasma-derived components may be infected., it is still a threat in developing countries(8).

If HBsAg persists for more than 6 months, spontaneous clearance is very improbable and infected individual is considered a chronic HBV (carrier)(9). Blood that is free of HBsAg but has titer antibodies against hepatitis B core antigen (anti-HBc) in the absence of antibodies against hepatitis B surface antigen(anti-HBs) may be also transmit HBV infection(9,10).

The HBcAg is not detectable in blood stream, But anti-HBc are first detectable antibodies to appear in serum shortly after the appearance of HBsAg in acute HBV infections (around 8 weeks) (3). It will persist after the disappearance of HBsAg and before the appearance of detectable anti-HBs(11)._The people anti-HBc with anti-HBs may have resolved infection, leaving the person immune(12). The presence anti-HBc has been used to determine if the presence of anti-HBs was due to vaccination or by previous exposure to the liver virus (13).

The Architect system is intend to be used as a screen for blood to prevent transmission of HBV to recipients and aid diagnosis of HBV infection.

Expanded blood donor selection procedure and improved laboratory detection of viral pattern have reduced the risk of transfusion transmitted viral infection. We conducted this study to investigate the distribution of anti-HBc and other HBV patterns in apparently healthy Iraqi blood donors who were HBsAg negative and to determine the major independent risk factors beyond HBV exposure with the goal of improving blood donation safety by using Architect System.

Materials and methods:

Between July 18, 2011 to December 25, 2011, 10ml of blood samples were collected from 25782 Iraqi's blood donors in National Blood Transfusion Center (NBTC) donors, Baghdad, 25294 of them donors were male and 488 were female, mean age (20-65)years.

All samples were tested using Enzyme-linked immunosorbent

**Dept. of biology, College of Education for pure Sciences/Ibn Al-Haitham/ Baghdad University.*
*** *Dept. of biology, National blood Transfusion Center.*
zainab_biol@yahoo.com

assay (ELISA) for detection of HBsAg in blood donors according with the manufactor's instruction Plasmatec company, UN. code no.2011100903. HBsAg positive sera were excluded (185) and final number of donors included in the study was 25597 HBsAg negative blood donors. Then, determined of anti-HBc (Total: IgM and IgG) in HBsAg negative sera donors, Baghdad by using a chemiluminescent microparticle immunoassay (CMIA) technology (Architect system) with flexible assay protocol, referred to as chemiflix Abbott company, Germaney, code no.14128L100. The result showed 1000 samples from 25597 were anti-HBc positive. Then, determined of anti-HBs(Abbott company, Germaney, code no. 25253LF00) for the sera that showed positive result for anti-HBc (NO.1000) with same technique.

Principle of architect system: specimen diluents and rHBcAg coated paramagnetic microparticles are combined. Anti-HBc present in sample binds to the rHBcAg coated microparticles and the reaction particles is washed. Then, anti-human acridinium-labeled conjugates is added. Follow another wash cycle, pre-trigger and trigger solution is added. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-HBc in the sample and the RLUs detected by Archetect system optic.

Serum samples which give HBsAg negative and anti-HBc positive stored at $(-40)^0$C and then thawed once for serological examination anti-HBs. Levels of anti-HBs were expressed in IU/ML subjects were classified according anti-HBs titer in 4 sub groups, sero-negative with anti-HBs levels> 10 IU/ML, sero-positive(sero-protective)< 10 IU/ML. Those with anti-HBs level >100 IU/ML were rated as having a low immune response and those with anti-HBs levels <100 IU/ML were rated as having a good immune response, who are supposed to be protected against HBV(14).

Results:

Exposure to HBV infection in 25782 Iraqi's blood donors: 25294 males, 488 females, mean age (20-65)years, Screen at various periods between 18 july 2011 to 25 December 2011 as shown in table: 1.

Table(1):Prevalence of HBV. infection In Iraqi blood donors

No. Blood donors		HBs-Ag Pos.[a] No.(%)	HBs-Ag Neg. No.(%)	Anti-HBc Pos.[b] No.(%)
Male	Female			
25294	488	185 (0.7%)	25597(99.3%)	1000 (3.8%)
Total: 25782				

[a]By ELISA+ [b]By Architect system=185+1000=1185 cases of infection

By ELISA: of the 25782 donors studied, 185(0.7%) were positive for HBs-Ag and 25597(99.3%) were HBs-Ag negative.

By Architect system: of 25597donors with HBs-Ag negative studied, 1000(3.8%) were positive for anti-HBc while the real result for HBs-Ag negative is 24597(95.4%).

Upon this study the rate of prevalence of HBV infection among Iraqi donors is 4.6%(No.1185).

The possible transmission of HBV by HbsAg- negative . This study showed that detection of HBsAg assay only less safe for the potentially HBV infection donation in Iraq (p>0.05) compared with other serological (anti-HBs with anti-HBc). A high numbers of false negative (No.1000, 3.1%) was observed in this study.

Table (2) HBV serological patterns in chronically blood donors by Architect system

Variables		NO.	%	P Value
HBV patterns[a]				
anti-HBs with anti-HBc(+ve).		685	68.5%	p>0.01
anti-HBc (+ve) alone[b]		315	31.5%	
Anti-HBs levels[c]				
Anti-HBs(+ve)10<100		472	47.2%	p>0.01
Anti-HBs (+ve) 100<1000		96	9.6%	
Anti-HBs (+ve) <1000		117	11.7%	
Gender[d]	Anti-HBc alone in male	311	31.1%	p>0.001
	Anti-HBc alone in female	4	0.4%	
Age group[e]	Age > 30	216	21.6 %	p>0.001
	Age < 30	784	78.4 %	

[a]Total NO.= 1000,[b] Level of anti-HBs negative10<,[c] Total NO.=615, [d]Total NO.=315, [e]Total NO.=1000

In the present study: Among 1000 cases with past HBV infection (chronic), anti-HBc (Total: IgM and IgG) with anti HBs were seen in 685 (68.5%) cases (Immunity cases). Anti-HBc (Total: IgM and IgG) alone were seen in 315 (31.5%) cases (Dangerous cases). This sera pattern have different significant (p 0>01).

But 685 (68.5%) cases have different titers of anti-HBs between more than 10IU/ml and more than 1000 IU/ml (p> 0.01). Most of the exposed group were male, the study present (311/1000) case for anti-HBc (Total: IgM and IgG) alone in male, But (4/1000) cases for that last in female. Male gender was a significantly association with past HBV infection (p>0.001). The age above thirty was the most significant for prediction of anti-HBc (Total: IgM and IgG)positive of blood donors (p>0.001).

No stablishing significant association between risk factors of exposure HBV., Excepted married, residence, and history of blood transfusion as shown in table(3).

Table (3)[A] Demographic charecteristics and clinical history as risk factors to exposure of HBV infection.

Risk factors		Exposure		Not exposure		P Value
		No.	(%)TotalNo.=1185	No.	(%)Total No.= 2000	
Martial status	Single	180	15.2	700	35	P<0.01
	Married	990	83.5	1231	61.5	
	Divorced	7	0.6	35	1.8	
	Widowed	8	0.7	34	1.7	
Residence	Rural	499	42.1	621	31	P<0.01
	Urban	686	57.8	1379	69	
Education	Illitrerate	290	24.5	410	20.5	
	Read and write	119	10	109	5.5	
	Primary	125	10.5	132	6.6	P<0.05
	secondary	191	16	344	17.2	
	University	460	38.9	1005	50.2	

Table (3) [B] Demographic charecteristics and clinical history as risk factors to exposure of HBV infection

Risk factors		Exposure		Not Exposure		P Value
		No.	(%)TotalNo.=1185	No.	(%)TotalNo.= 2000	
History of blood transfusion	Non	901	76	1700	85	P<0.01
	Infrequent	100	8.4	230	11.5	
	Frequent	184	15.5	70	3.5	
Undergone dental manipulation	Non	429	36.2	983	49.2	P<0.01
	Infrequent	416	34.9	677	33.9	
	Frequent	340	28.8	340	17	

Discussion:

Occult hepatitis B infection(OBI) is defined as persistent detectable viral genome in serum or liver while HBsAg is undetectable. The frequency of this condition is directly dependent on the sensitivity of assay for either or both HBV patterns (10). In Iraq, HBs-Ag screening by ELISA is only mandatory test for detection of HBV infection in blood donors. Ataallah (2011) present that 0.6% only of blood donors were positive for mandatory (traditionally) HBsAg only by ELISA(12). Characteristics of architect system: Architect system can detect HBs-Ag levels less or equal to 0.4ng/ml, Possibility to work on batch up to 200 tests per hour, Closed system, Reduce cost and time, Detected the most commen known mutants to ensure safe transfusion, Control per run: 2 every 24 hrs., Auto repeat and have touch screen. The Architect system are state of the art analytical systems that is providing a reliably result to detect HBsAg, anti-HBc and anti-HBs until in dry blood spots with comparative low analytical sensitivity in the other systems(15). Architect is a fully automated HBsAg assay system for a better detection of HBV infection than any the comparator assay and equivalent specifity for the detection and management of HBVinfection(16). Our study showed that 1000/25597(3.8%) cases of HBV infection diagnosed by Architect system, through anti-HBc assay in HBs-Ag negative donors, in addition to 185 cases by ELISA. The total no. of infected cases is 1185 out of 25782 (4.6%). The prevalence of chronic HBV infection varies according geographic regions, from high(8%>), intermediate(2-4%) to low2% (<) (17). The prevalence of HBV in Iraq is 2% according WHO (18). Due to few the a previous studies in Iraq by Architect system, So Iraq is consideded as a region of intermediate prevalence for HBV infection according this report (4.6%) compared with report Ataallah (2011) by HBsAg only as low region (12). Study in eastern india on 1027 HBsAg negative donors, It is found

that 188 (18.3%) of them were anti-HBc positive that carried out by PCR(19). These numbers refer to an underestimated rates of exposure to HBV infection among Iraqi blood donors. Therefore, we must reduce this rate to zero by using this technique as first step to safe blood transfusion. The prevalence of HBV infection among Italian first time blood donors is much lower than in the past by using commercial immunoassay(20).

Screening of anti-HBs also performed on all anti-HBc positive sample (NO.1000, 3.8%). This study showed increased of pattern serum anti-HBc with anti-HBs in rate 68.5% comparasim to in 31.5% of anti-HBc alone ($p>0.01$). This study is consistent with El-Ghitany and Farghaly that refered to pattern anti-HBc with anti-HBs was present in almost half of those showing anti- HBc alone(3). It can benefit from first in protection from HBV infection (leaving person immunity). In addition, the individual with this condition and who are anti-HBsAg negative with anti-HBc negative should be selected as a regular blood donors to safe blood transfusion of Infection. While, second suggestion is to prevent individuals with sera patterns anti-HBc alone from donating blood again to minimize transmission occult HBV. Although, It may lead to the exclusion of significant number of blood donors. For comparison, studies performed in Iran, prevalence of anti-HBc alone in patients with past infection was 3.9%(21), in Lebanese decreased to 2.2% blood donors were anti-HBc alone (22). Refers Niederbauser et al. to low rate anti-HBC to 2% by Nucleic acid testing(NAT) (23). Similarly, anti-HBc alone was reported in 2.1% of Saudi blood donors(24). As reported in Brazil where high levels of anti-HBc were recorded up to 57%(25). There are several reasons which may explain the differences in anti-HBc prevalence, such as a preselected donor population, different screening and confirmation algorithms and regional differences (14). But, anti-HBs were positive in 685/1000(68.5%), they have different titers ranging from more than 10 LU/ml to more than 1000 IU/ml ($p>0.01$). The study showed high levels 10100> IU/ml in donors Iraqi (47.2%) . Our findings agree with Niederauser et al.(23), and Bahatti et al.(21). The investigators stressed that blood components positive for anti-HBc with anti-HBs do not appear to transmit HBV and there is clearly an inverse correlation between anti-HBs level and infectivity(3). The high rates of antibody persistence could be secondary to natural boosting from unapparent viruses or antigen from infected persons (24). The different titer of anti- HBs positive donors sera can be providing protecting against of HBV Infection. Chen and Oon refered reported that a dose of anti-HBs above 10 IU/ml are regarded as sufficient for protection against HBV infection(14). The present study and several publisher concluded that male gender play an important in acquisition of HBV infection (13, 14, 25). But, one study releaved that the gender is significant important in female compared with unexposed group (2). Older age (age< 30) was significantly associated with the detection of anti-HBc alone as factor transmitted to infection ($p> 0.001$) as shown in table (2). Similarity, Said et al. reported that the age above thirty were the most significant risk factor for prediction anti-HBc positive among blood donors (1). Other authers also showed anti-HBc prevalence increased with age, suggesting that sexual activity may contribute to transmission of this infection among adult(2).Young blood donor in Pakistan are already more for HBV exposure (21). This significant association of HBV infectprone with older age could also due to the increased number of years of exposure, lack awareness and infrequent HBV vaccination in adults(27). The other risk factors were Neither showed in this study any association with ABO and Rh blood groups compared their with non exposure $P> 0.05$ ($X^2=6.676$ for ABO, $X^2=2.13$ for Rh). Recommendation: Inserted serological patterns anti-HBc and antiHBs assay in all centers of blood transfusion in Iraq. Still our ambition is more and hope some day use nucleic acid test in our investigation. Acknowledgements: Our thanks go to all of the blood donor participant involved, as well as to the National blood Transfusion Center (NBTC) team.

Author contributions:

study of design: Zainab S. Hassab and Yakub A. Saleh. Study conception:. Amani, K. Abd l-Rhman and Auroba I. Abd-Allah Interpretation of data: Zainab S. Hassab, Drafting of manuscript: Zainab S. Hassab.

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