Clinical and prognostic values of anti-hepatitis B core immunoglobulin M detection in asymptomatic hepatitis B surface antigen carriers

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ABSTRACT

Objective: The aim of this study is to evaluate the clinical and prognostic values of persistence of anti-hepatitis B core immunoglobulin M antibody in asymptomatic adults chronic hepatitis B surface antigen anti-hepatitis Be carriers and its absence in others.

Methods: Fifty-two hepatitis B surface antigen/anti-hepatitis Be carriers with and 32 without anti-hepatitis B core immunoglobulin M marker were enrolled in this study. The cases were regular attendees of Public Health Laboratory, Virology Center (the main referral center for viral hepatitis) Mosul, North Iraq, for follow-up and clinical evaluation. The study was performed from June 1999 to June 2001. The studied groups consisted of adults, with mean age of 35.5 year (standard deviation $\pm$ 10). The results of histological findings of 23 carriers with and 12 carriers without anti-hepatitis B core immunoglobulin M who underwent liver biopsy were added to the study. Micro enzymed-linked immunosorbent assays was utilized to detect hepatitis B virus markers.

Results: Existence of carrier in the family was significantly associated with persistence of anti-hepatitis B core immunoglobulin M in the studied individuals ($p<0.005$, odds ratio = 7.4; 95% confidence interval = 1.8 to 38.0), as was the case in the presence of family history of acute hepatitis ($p<0.05$, odds ratio = 4.6; 95% confidence interval = 4.6 to 21.2). The detection of this antibody was significantly associated with the presence of abnormal liver histology compared to carriers without this antibody ($p<0.01$, odds ratio = 7.2; 95% confidence interval = 1.8 to 28.7). The study revealed that clustering of carrier cases existed in statistically significant ($p<0.001$) pattern in family members of carriers with anti-hepatitis B core immunoglobulin M.

Conclusion: The detection of anti-hepatitis B core immunoglobulin M clinically is a reminder of recent exposure to the virus through different routes, mainly intrafamilial. Ongoing liver changes are observed in the majority of carriers with this antibody indicating the viral activity, albeit in a silent manner, but earlier progress to serious liver sequels may be inevitable. Foretelling that carriers with anti-hepatitis B core immunoglobulin M are more infectious than carriers without this marker is ascertained by the existence and clustering of carrier cases amongst their family members.

Keywords: Hepatitis B surface antigen carriers, anti-hepatitis B core immunoglobulin M, liver biopsy.

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The discovery of the safe and immunogenic hepatitis B vaccine 2 decades ago tempered the claims for reaching total eradication of hepatitis B viral infection as a serious infectious disease. The

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emergence of certain pitfalls during vaccine usage, with the absence of effective treatment of acute and chronic cases, and inability to clear the virus from carriers impeded eradication and made it an unrealistic prospect in the near future.[1,2] The policy of a systematic follow up program of all chronic hepatitis B surface antigen (HBsAg) carriers would determine the infectivity of the subjects, the burden of liver disease in the country, the most possible routes of infections, and the appropriate methods to be adopted to reach the goal of eradication. Chronic hepatitis B cases after acute infection vary as a function of age. In neonates, the majority (>90%) of acute infections end up with chronic carriers. However, in adults, most acute infections resolve, only 3-5% of cases remain chronically infected.[3,4] Depending on the relative level of replicative hepatitis B virus (HBV) replication, chronic hepatitis B could be subdivided into 2 phases. Firstly, replicative phase, which is characterized by the presence of hepatitis Be antigen (HBeAg), HBV-DNA, high infectivity, and liver damage. Secondly, the non-replicative phase, which is characterized by the presence of anti-hepatitis Be (anti-HBe), undetectable HBV-DNA in the serum (integrated into hepatocyte), limited infectivity, and normal or minimal liver injury.[5] In most instances, HBV is not cytopathic, the host immune response directed to infected hepatocyte is vital to produce liver damage and viral clearance.[6] The main immune response is mediated by cellular response to hepatitis B core antigen (HBcAg), presented on the surface of infected hepatocytes through human leukocyte antigen (HLA) class I-restricted cytotoxic cluster of differentiation (CD) 8+ cells leading to direct cell killing and subsequent liver damage.[7,8] Antibodies to hepatitis B core antigen (HBcAg) are regularly produced during hepatitis B viral infection and are sensitive indicators of viral replication.[9,10] Initially the antibody is of immunoglobulin M (IgM) subtype, and different studies have already confirmed that anti-HBc IgM always develop during the acute stage of HBV infection.[11,12] This antibody disappears within 4 to 8 months after the acute stage. This antibody then will be replaced by IgG subtype that may persist life long as proof of previous hepatitis B viral infection.[13] Recently, with the use of highly sensitive and specific serological assay methods, persistence and variations in the kinetics of anti-HBc IgM marker are frequently and surprisingly observed during the chronic hepatitis B infection. Its persistence was registered in 20% of carrier cases even for more than 2 years after acute infection.[12] This study was designed to elucidate the clinical and prognostic significance values of detection of anti-HBc IgM in apparently healthy HBsAg/anti-HBe positive carriers and its absence in others.

Methods. Fifty-two HBsAg/anti-HBe carriers with anti-HBc IgM and 32 without anti-HBc IgM markers were enrolled in this study. The cases were regular attendees of the Public Health Laboratory, Virology Center (the main referral center for viral hepatitis), Mosul, North Iraq, for follow-up and clinical evaluation. The study was performed from June 1999 to June 2001. The subjects were eligible for the study if they fulfilled the following criteria: known HBsAg/anti-HBe carrier for more than 6 months with or without anti-HBc IgM, asymptomatic, with normal liver chemical tests mainly the aminotransferase enzymes (ALT) and with no evidence of any immunodeficiency diseases or states. The studied groups consisted of adults, with the mean age of 35.5 year (standard deviation [SD] +10). Liver histology results of 23 with anti-HBc IgM and 12 without anti-HBc IgM participants who were underwent biopsy, either prior to the study or during the study period by their physicians to confirm the liver histology, were included in the study. The carriers were interviewed and information about some possible recognizable risk factors for acquisition with hepatitis B viral infection were recorded using a questionnaire which included: history of acute hepatitis of recent onset, family history of jaundice or existence of carrier case, parenteral exposure through blood transfusion (BT) or frequent intravenous injections, and type of occupation. Screening of carrier contacts was a routine policy and the result of screening of family members of both groups of carriers were added to the study (children under 12 years were not included since most were vaccinated). The study utilized by Biotest Kits (Germany) for HBsAg and Hepanostika Kits (Organon Teknika, Netherlands) for HBeAg, anti-HBe, anti-HBc IgM, and total anti-HBc. Superinfection with Delta hepatitis and hepatitis C viruses were excluded by appropriate serologic testing. Statistical analyses, such as odds ratios (OR), 95% confidence interval (CI), and p values were estimated using 2x2 contingency tables.

Results. **Table 1** shows the correlation of some possible risk factors in acquisition with hepatitis B viral infection and subsequent carrier state development in the studied groups. Existence of carrier in the family was significantly associated with persistence of anti-HBc IgM in the studied carriers ($p<0.005$), as was the presence of family history of acute hepatitis ($p<0.05$). No significant associations were detected in regard to the presence or absence of history of acute hepatitis, BT, frequent intravenous injections, or performing at risk occupations. Moreover, carriers with a family history of acute hepatitis and existence of carrier in the family were at increased risk for persistence of anti-HBc IgM marker compared to others without this antibody (OR=7.4; 95% CI=1.8 to 38.0 and OR=4.6; 95%

CI=4.6 to 21.16). History of acute hepatitis and frequent intravenous injections were found to carry an additional risk for persistence of this antibody (OR=3.5; 95% CI=8.4 to 29.4 and OR=5.6; 95% CI=1.4 to 44.26), as was BT and risky occupation (OR=2.6; 95% CI=4.2 to 28.3 and OR=2.6; 95% CI=4.2 to 28.3). The results of histologic findings are shown in **Table 2**. In carriers with anti-HBc IgM, the overall rate of observed abnormal liver histology was 78.3% (minimal changes in 52.2% and chronic persistent hepatitis (CPH) in 26.1%). On the other hand, in carriers without this antibody minimal changes were the only abnormal histology detected in 33.3% of cases. Therefore, persistence of anti-HBc IgM was significantly associated with existence of abnormal liver histology compared to carriers without this antibody ($p<0.01$) and carry increasing risk of developing serious liver damage (OR=7.2; 95% CI=1.8 to 28.7). **Table 3** shows HBsAg status amongst screened family members of both groups. The study revealed that clustering of carrier cases do exist in statistically significant ($p<0.001$) pattern amongst contacts of carriers with anti-HBc IgM compared to carriers without this antibody. Anti-HBc IgM was detected in 46.3% of family carriers of carriers harboring this antibody, while it was absent amongst family carriers of the next group.

Discussion. In immunocompetent individuals the immune response to HBV depends on the match between HBV peptides presented by the host major – histocompatability - complex molecules and the specific T-cell-receptor repertoire of the host.[13] This immune response may clear the infection by destroying all infected hepatocytes and aborting viral replication, if sufficient recognition and activation occur. If the response is inadequate, the infection continues to chronic stages. This study was performed on a group of asymptomatic HBsAg/anti-HBe carriers and indeed all of our patients belong to the non-replicative phase or the 3rd stage of life cycle of HBV infection. In this stage, HBeAg was no longer present, and anti-HBe became detectable with marked decrease in viral DNA with marked fluctuation in HBV-DNA as detected by polymerase chain reaction (PCR) and ALT returned to normal levels.[13] Anti-HBc IgM was always detected after exposure to HBV and its association with acute HBV infection was documented. The significance of anti-HBc IgM persistence in chronic cases was not easily understood. In this study, some of our carriers with anti-HBc IgM (**Table 1**) had a recognizable history of exposure to HBV. The infection may either follow acute icteric stage or after an inapparent acute hepatitis B viral infection through different exposure routes. Existence of carrier in the family was significantly associated with persistence of anti-HBc IgM in the studied carriers, as was the presence of family history of acute hepatitis. However, in carriers without anti-HBc IgM the majority have no recognizable risk factors and they represent mostly an early age exposure. This might be either through perinatal or postnatal horizontal transmission with persistence of infection into adulthood. Such carriers as they grew and marry, remained as the orphan cases within their new family (**Table 1**).[14,15] In view of this study, the explanation for the persistence of this anti-HBc IgM in some convalescence carriers, after acute stage of infection, might be attributed to the continual contact of the immunocompetent cells with the

Table 1 - Possible risk factors for acquisition of HBV in HBeAg/Anti-HBe carriers with or without anti-HBc IgM.

Risk factors	HBsAg/Anti-HBe carriers				OR (95% CI)	P-Value
	With anti-HBc IgM N=52		Without anti-HBc IgM N=32			
	Present	Absent	Present	Absent		
History of acute hepatitis	5	43*	1	30	3.5 (8.4 to 29.4)	0.25
Family history of hepatitis	13	35	2	25	4.6 (4.6 to 21.2)	0.05
Existence of carrier in family	17	30	2	26	7.4 (1.8 to 30)	0.005
Blood transfusion	4†	48	1	31	2.6 (4.2 to 28)	0.5
Frequent intervenous injections	8‡	44	2	30	5.6 (1.4 to 44.3)	0.1
Risky occupation	4§	48	1	31	2.6 (4.2 to 28)	0.5

N - number, OR - odds ratio, * - some do not answer all questions, † - unscreened blood given on emergency basis outside the city, ‡ -IDDM and asthmatic cases, § - hospital workers, HBV - hepatitis B virus, HBeAg/Anti-HBe - hepatitis Be antigen and anti-hepatitis Be, IDDM - insulin dependent diabetes mellitus

Table 2 - Histological findings in liver biopsy performed from 23 carriers with and 12 carriers without Anti-HBc IgM marker.

Histological liver changes	HBsAg/Anti-HBe carrier	
	Anti-HBc IgM positive N (%)	Anti-HBc IgM negative N (%)
1. Minimal changes	12 (52.2)	4 (33.3)
2. CPH	6 (26.1)	0 (0)
3. CAH	0 (0)	0 (0)
4. Liver cirrhosis	0 (0)	0 (0)
Total	**18 (78.3)**	**4 (33.3)***

N - Number, *p< 0.01, OR and 95% CI = 7.2 (1.8 to 28.7), CAH - chronic active hepatitis, CPH - chronic persistent hepatitis, Anti-HBc IgM - anti-hepatitis B core immunoglobulin M, HBsAg/Anti-HBe - hepatitis B surface antegin/anti-hepatitis Be, OR - odds ratio, CI - confidence interval

Table 3 - Hepatitis B surface antigen status in family members of HBsAg carriers with or without anti-HBc IgM (children under 12 year old were not included).

Studied HBsAg carriers (N)	Screened corresponding family members			
	N	Mean age year ± SD	HBsAg positive	
			N (%)	With anti-HBc IgM (%)
With anti-HBc IgM (52)	220	32 ± 8	41 (18.6)	19 (46.3)
Without anti-HBc IgM (32)	128	31 ± 9	5 (4.2)	0 (0)

N - Number, *p< 0.01, OR and 95% CI = 7.2 (1.8 to 28.7), HBsAg - hepatitis B surface antigen, Anti-HBc IgM - anti-hepatitis B core immunoglobulin M, OR - odds ratio, CI - confidence interval

HBcAg presented on the surface of infected hepatocytes. Moreover, this antibody may reappear during flares or reaction of their existing chronic viral infection. Periods of hepatic exacerbation or silent forms of type 2 chronic hepatitis B (mutant forms), or both, in some asymptomatic HBsAg anti-HBe carriers may occur with the detection of anti-HBc IgM. It was suggested that chronic asymptomatic carriers with normal liver chemistries need not to undergo liver biopsy, specially if they were HBsAg/anti-HBe carriers. In fact from literatures the overall incidence of chronic hepatitis among these subjects seemed to be very low[16-18] However, in hepatitis B viral infection the liver histologic changes did not directly mirror the virologic stage of disease predicted by serologic markers, therefore, some physicians attempt to perform liver biopsy to determine the pattern of liver histology. In this study, albeit the number of liver biopsy underwent was low, the persistence of anti-HBc IgM was significantly associated with existence of abnormal liver histology compared to carriers without this antibody and carry increasing risk to progress to serious liver damage later in life (**Table 2**). Those carriers with anti-HBc IgM and evidence of liver damage represent a state of viral activity or flare up of their disease process with an ongoing silent infection (asymptomatic and normal ALT levels). Although minimal liver changes were detected in most cases with anti-HBc IgM who underwent liver biopsy but CPH was observed in 2 blood transfused, 2 insulin dependent diabetes mellitus (IDDM), and 2 asthmatic patients. It was documented that the course of chronic hepatitis B was more serious in accidentally or unscreened blood transfused patients or in drug addicts.[19,20] This study also revealed statistically significant existence and clustering of carrier cases amongst family members of carriers with anti-HBc IgM compared to family members of other studied carrier groups (**Table 3**). The tendency for family clustering of HBV carriers with liver disease had been noted previously.[21] Moreover, anti-HBc IgM was observed only in family carriers of cases with this antibody and was absence in others. In this country, with screening of all blood donors and absence of drug addicts and abnormal sexual behavior, the most important way of hepatitis B viral infection spread is horizontal intrafamilial route.[14,15]

In conclusion, the presence of this antibody indicates a slow viral activity and the ongoing liver damage either due to continual viral activity from acute stage or reaction of their existing disease with a silent ongoing infection. Polymerase chain reaction study and DNA hybridization methods are recommended to study the nature and genome sequences of the virus in such carriers to exclude emergence of mutant strain of HBV in quasi species. It is prudent to subdivide anti-HBe carriers into those with or without anti-HBc IgM, such sorting is necessary and may improve the serodiagnostic accuracy of hepatitis B viral infection and having clinical and prognostic values. In such carriers with ongoing liver damage a remedy of the problem is needed. The use of antiviral drugs, such as HBV replication inhibitors, in chronic carriers with HBeAg and progressive liver damage need to be tried in this situation.

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