Kufa Medical Journal | Vol. 19, No. 1, 2023

Original article | DOI: https://doi.org/10.36330/kmj.v19i1.12180

Submitted at: 26 May 23
Accepted at: 06 June 23

The Impact Of Hepatitis B Infection On The Development Of Anti-Cardiolipin Antibodies In Najaf Province

Ameer Mohammed Kadhim [(1)], Hawraa Ameer Mubark[(2)], Dhefaf Hameed Al-Mudhafar[(3)], And Saif Jabbar Yasir[(4)]

[(1)] B.sc in Medical lab technologies /Medical Microbiology, Faculty of Medicine, University of Kufa, Najaf, Iraq.

[(2) (3) (4)] Ph. D. in Medical Microbiology, Faculty of Medicine, University of Kufa, Najaf, Iraq.

Corresponding Author Email: ameerm.aIessawi@student.uokufa.edu.iq

Abstract

Background: Hepatitis B Virus (HBV) is a partly double-stranded, hepatotropic, non-cytotoxic Hepadnaviridae virus. HBV is linked to many autoimmune diseases, including Rheumatoid Arthritis(RA), Cryoglobulinemia, and Antiphospholipid Syndrome(APS). Anticardiolipin antibodies (ACL) are a type of anti-phospholipid antibodies(APA) that target cardiolipin molecules. They're more common in systemic lupus erythematosus (SLE) and APS. APA may be caused by viral infections such as HBV. **Aim of the study:** Determination of Anticardiolipin antibody positivity among hepatitis B virus-infected patients in Najaf governorate. material, and methods: a cross-sectional study was conducted between September 2022 to March 2023 in Najaf, a non-random sampling was depended, the sera of 113 HBV-infected patients were collected from the main health facilities in Najaf, and all had no other possible disease that is associated with APA, then it was tested for HBS antigen, total HBc antibodies, IgM HBc antibodies, ACL antibodies by ELISA technique. SPSS version 26 was used to perform the statistical analysis processes. **Results:** anti-cardiolipin antibodies(ACL) prevalence was **10.6%**, The type of HBV infection and ACL production were significantly correlated ($p<0.05$), males had a higher percentage of ACL autoantibodies and middle age patients presented with the highest rate of ACL antibodies, however, there was no statistically significant relationship between ACL and age or gender. In **Conclusion:** hepatitis B virus (HBV) is one of the main viruses that lead to the production of ACL especially in middle-aged people males, the chronic type is more prevalent for ACL than the acute type.

Keywords: HBV, ACL, Najaf, Cross-Sectional, ELISA

INTRODUCTION

Hepatitis B virus (HBV), a partially double-stranded DNA-enveloped virus, is a common human disease-causing virus [(1)]. HBV is a hepatotropic, non-cytopathic virus that has the capacity to infect individuals for a long time [(2)]. HBV uses numerous mechanisms to exploit host innate immunity in order to increase its proliferation[(3)]. Despite being generally a self-limiting infection, Acute HBV infection might leave residuals that may become active under immunosuppressive circumstances. Hepatic fibrosis is frequently linked to the persistent manifestation of viral hepatitis, leading to

advanced liver ailments such as cirrhosis, hepatic insufficiency, and carcinoma in certain individuals.[4]. The abrupt increase in hepatitis B virus (HBV) replication in a patient with dormant or treated hepatitis B is known as hepatitis B reactivation. Reactivation can occur naturally, although it is more frequently brought on by immunosuppressive drugs used to treat cancer, autoimmune diseases, or organ transplants [5]. The level of reactivation varies greatly depending on the patient, the virus, and the treatment. HBV reactivation can cause moderate hepatitis to severe bouts that might result in abrupt liver failure and death[6].

Anticardiolipin antibody (ACL) is a type of autoantibody that belongs to the antiphospholipid antibody (aPL) family. ACL target antigens are phospholipids with a negative charge on platelets and endothelial cell membranes [7].there are three types of anti cardiolipin antibodies (IgA, IgM, IgG), It has been linked to many disorders including autoimmune disease, recurrent abortion, cerebrovascular disease, and infectious disease. Phospholipid-binding protein is the target antigen of ACL in autoimmune disease and antiphospholipid syndrome (APS), which can result in coagulation disorder; cardio phosphate is the target antigen of nonimmune ACL found in syphilis and other infectious diseases, which does not rely on plasma proteins like B2GPI. [7].ACL was significantly elevated in certain viral diseases [8] ,even transient ACL may be a biomarker for development of Myocardial infraction in patients with atherosclerosis(20).

Multiple studies showed different results regarding HBV and Antiphospholipid antibody(APLA) ,APLA positivity and thrombotic problems linked to antiphospholipid antibody are both highly correlated with viral hepatitis [9, 10].others were on opposite side , With HBV infections, ACLA has no pathogenic importance. [11]

This study aims to determine the percentage of anti-cardiolipin antibodies among the HBV infected patients who neither had current nor previous other possible reason for ACL positive result other than HBV.

MATERIAL AND METHODS

Study design and sample collection

This study is cross-sectional type, which was held in Najaf governorate in the time between(September 2022 to march 2023) , a non-random sampling method was followed depending on the exclusion criteria , it included a total number of one hundred and thirteen patients (71 were males),age range was (15-85 years(yrs.)., median was 43 yrs.), they were attending the specialized center for gastroenterology and hepatology in Najaf province and the public health laboratory, they were either referred by Gastrointestinal Tract (GIT) specialist clinics or by routine checking (during the routine checking prior to blood donation, contact with infected subjects with HBV, preparation for surgery) ,none of them had previous history of (systemic lupus erythematosus (SLE), deep venous thrombus(DVT) , anti-phospholipids syndrome (APS) ,multiple miscarriages as for females), prior to the sample collection a verbal consent was obtained from the patients and from their parents for those who were younger than 18 years old.

Serum sample processes and ELISA Techniques:

Five milliliters of venous blood samples were collected into gel tube from each patient, allowed to clot and then the serum sample was obtained by centrifugation and kept in plain tube to perform the required tests.

firstly, the sera of all 113 patients were positive for HBS antigen result using ELISA technique (kit manufacture by fortress/Ireland), the test procedure was according to the manufacture instructions and all positive results were confirmed by mini vidas .

Next, the serum of those 113 patients was placed into three Eppendorf tubes (one Eppendorf tube for each test) and stored in deep freezer (-80 c°) by (GFL/Germany) for later use. when sample collection completed, after that, the serum was checked for both total HBc and IgM HBc antibodies by qualitative ELISA technique, the kits manufactured by (Abia company/Germany and sun long biotech /china) respectively.

Then, anti-cardiolipin antibodies (ACL) (IgA, IgM, IgG) were detected by qualitative ELISA technique (sun long biotech, china). the cut-off value was (0.28) depending on the kits manual instruction, any sample with a result higher than the cut-off value was considered positive for ACL.

All test procedures were done according to manufacture's instructions provided by the kits manual.

Ethical approval

This study obtained the ethical approval from the internal ethical committee of the medical microbiology department/faculty of medicine / university of kufa and the health directorate in Najaf province, additionally, verbal consent was obtained by all of the included patients or their parents prior participation.

Statistical analysis:

Version 26 of SPSS was used for the statistical analysis (Inc. Chicago, IL, USA), chi square, median and Pearson correlation were applied.

RESULTS

All of the 113 patients tested positive for HBS antigen after duplicated ELISA test, which confirm their infection with HBV.

also, all of them were positive for the total HBc antibody, but only four patients (3.5%) were positive for IgM HBc antibodies (which indicates recent infection <6 months), those four were considered as acutely HBV infected and the rest (109) were considered as chronically infected with HBV.

Only 12(10.6 %) patients were positive for ACL, males presented with higher rates than females (8 positive cases were males), which can be seen in fig.1

Regarding their age groups, four patients (33.3%) were within the age group 41-50 yrs., then the age group (61-70 yrs.) came second and presented with 3 cases, the lowest ACL positivity rate was seen in age group>70 yrs. with no positive cases at all. Overall, anti-cardiolipin antibodies had no significant association with age groups as shown in tab. 1.

Regarding the type of HBV infection, most of the positive cases (9 out of 12) were chronically infected. Patients with chronic HBV infection had higher chance to develop ACL antibodies (any of the three types) than those with acute HBV infection , this is indicated by higher mean of ranks in tab. 3. There was non-significant negative correlation between age & ACL (N=113, P= .356, r^2= -0.008), this can be seen in fig.2. There was no significant difference in ACL regarding to gender, but there was a significant association between the type of infection with HBV and ACL, patients with chronic HBV infection had the higher possibility to develop ACL antibodies than acutely infected individuals. As shown in tab.2 and tab. 3.

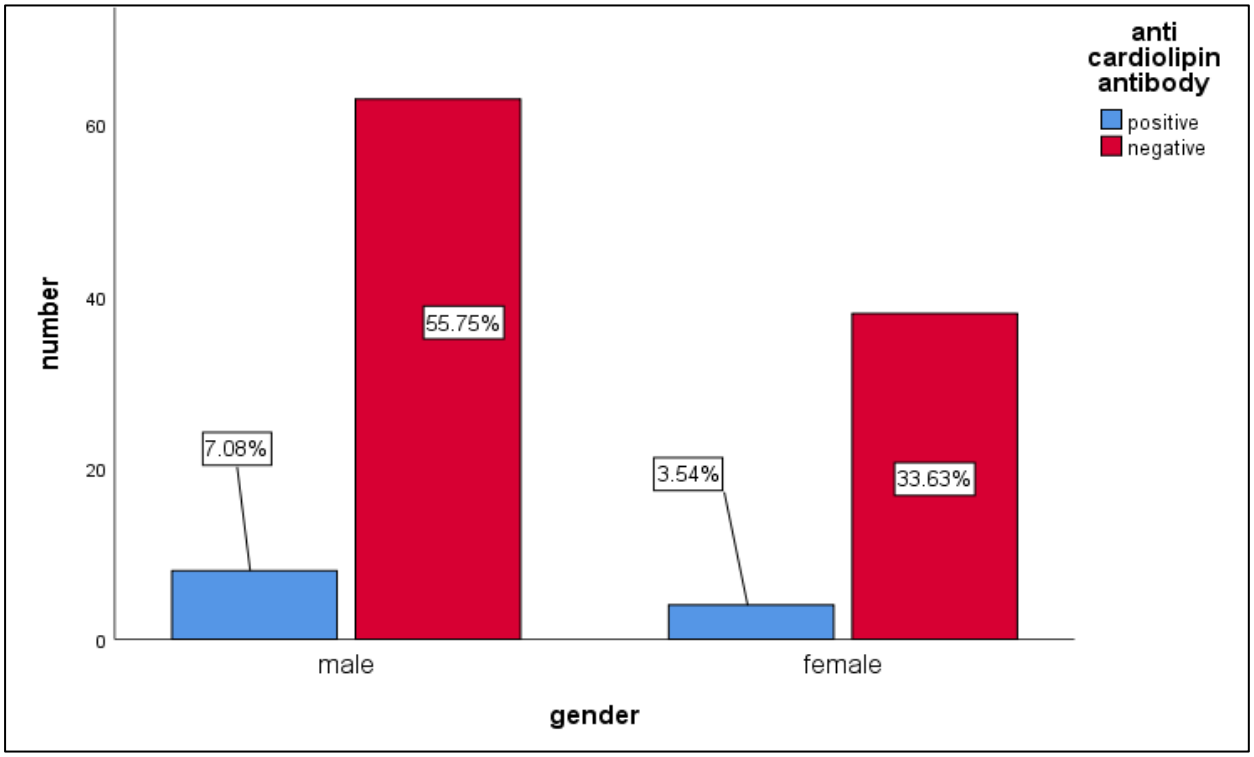

Figure No.1: distribution of anti-cardiolipin antibodies result according to the gender of the study patients.

Table No.1: distribution of ACL antibodies among HBV infected patients according to their age groups.

		Age (yrs.)							
		< 20	20-30	31-40	41-50	51-60	61-70	>70	
Anti cardiolipin antibodies	positive	1 (11%)	2 (11.7%)	2 (8%)	4 (12.9%)	0 (0%)	3 (30%)	0 (0%)	X^2=6.829
	negative	8 (88.%)	15 (88.2%)	23 (92%)	27 (87%)	10 (100%)	7 (70%)	10 (100%)	df=6
	total	9 (100%)	17 (100%)	25 (100%)	31 (100%)	10 (100%)	10 (100%)	10 (100%)	P=.337

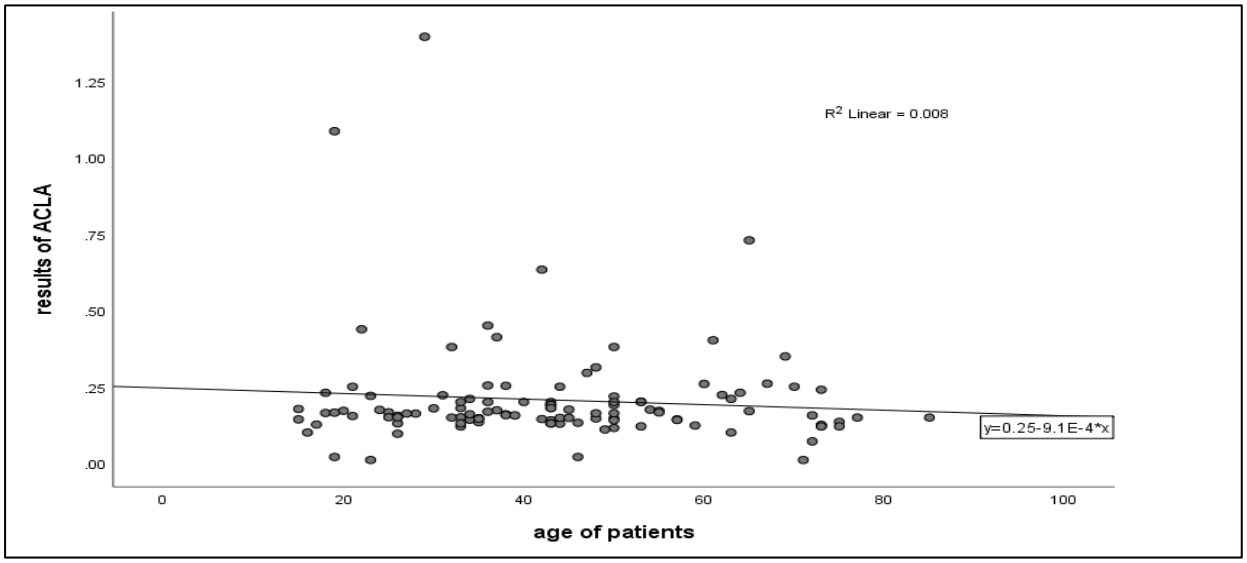

Figure No.2: Pearson correlation between the age of patients and anti-cardiolipn results obtained by ELISA

Table No.2: Distribution of anti-cardiolipin antibodies in HBV infected patents according to their gender and type of infection with HBV.

		Anti cardiolipin antibodies		
		positive	negative	
1-Type of HBV infection	Acute	3(25%)	1(1%)	X^2=18.108 Df=1 P=.0001
	Chronic	9(75%)	100(99%)	
	Total	12(100%)	101(100%)	
2-Gender	Male	8(66.6%)	63(62.3%)	X^2=.085 Df=1 P=.771
	Female	4(33.3%)	38(37.6%)	
	Total	12(100%)	101(100%)	

Table No.3: Mann Whitney test results between the type of HBV infection and anti-cardiolipin antibodies positivity.

	State of infection with HBV	N	Mean Rank
Anti-cardiolipin antibody	acute	4	20.63
	chronic	109	58.33
	Total	113	
Z= -4.237 , probability value(level of significance <0.05) :p value=000			

DISCUSSION

It is possible that ACL has a role in the thrombotic mechanisms that occur in advanced liver disease and HCC because it is present in a significant portion of patients with HBV-cirrhosis-related hepatocellular carcinoma (HCC) and peripheral venous thrombosis (PVT) [12]. Twelve out of 113 (10.6%) patients with HBV had produced ACL without any other possible cause such as SLE, DVT, etc. this percentage is considered lower than what was reported by previous studies where the range of people with hepatitis B virus who had ACL was between 14% to 42% [13, 14] [12] [15]. but it is almost the same as the Korean study by (Huh , et al) [16] where their ACL prevalence was 10.5% .this is maybe due to the higher cut-off positivity value in the current study than other studies.

The presence of ACL in patients infected with HBV or other viral infection may be explained by the disturbances in humeral and cellular immunity that occur in viral infection, Patients with persistent viral hepatitis may develop antibodies as a result of damaged liver cell membranes' triggering of neo-antigens. [17]. The alteration of plasma membrane phospholipids, as well as their overexpression on the apoptotic cell membrane, caused by viral apoptosis, may result in ACLA manifestation [14]. these antibodies may be transient and disappear later in life, or may progress to APS and other thromboembolic events such as DVT [18] [19]. Males dominated in the current study with higher ACL positivity frequencies than females, this is the same as it was reported by (Truglia S , et al) [20] where they reported males gender had a chance of 3 times in developing APS and associated thromboembolic events than females. Regarding the age groups , the findings are almost the same as (Huh, et al) [16] , the highest rate was seen in age group (41-50 yrs.) , and the lowest was seen at both groups (51-6- yrs.) and (>70 yrs.)

It also shown there was negative correlation between age and ACL levels Which means the level of ACL would decrease as age increase and vice versa, this is in agreement with the fact that APS occurs between age 20-50 yrs. rather than older age. Chronically infected patients experienced higher percentage for ACL positivity , which could be due to prolonged liver damage causes (e.g. cirrhosis) that led to the production of ACL antibodies over the time . As for the acutely infected patients also had their chance of ACL positivity, The endothelium of the portal vein system, in particular, might experience inflammatory alterations brought on by acute viral hepatitis, which can activate the coagulation system as a result of the inflammation and increase the risk of portal vein thrombosis[22]. Because a repeated positive result for ACL with 6-12 weeks' interval is required to confirm the diagnosis[23] we couldn't confirm the presence/absence of APS in those patients, .

CONCLUSION

The current study showed that:

1-Patients with HBV infection may develop ACL antibodies without the need for presence of any other secondary diseases. 2-Males with HBV have higher chance to develop ACL antibodies than females, its development is more likely in people who are younger. 3-chronically infected people with HBV are more likely to produce autoimmune antibodies. But, acutely infected patients might also develop ACL antibodies .

ACKNOWLEDGMENTS

I would like to express all my gratitude for the head and staff of medical microbiology department in faculty of medicine/ university of Kufa . Also to Prof. Dr Abdulkareem Mahmood in community medicine department / faculty of medicine / university of Kufa and Prof. Dr. Thikra Mahmood in medical microbiology/faculty of medicine/university of Kufa for the big help in statistical analysis .

REFERENCES

1. Herrscher C, Roingeard P, Blanchard E. Hepatitis B Virus Entry into Cells. Cells. 2020;9(6):1486.
2. Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. Nature Reviews Immunology. 2022;22(1):19-32.
3. Tsai K-N, Kuo C-F, Ou J-HJ. Mechanisms of Hepatitis B Virus Persistence. Trends in Microbiology. 2018;26(1):33-42.
4. Noordeen F. Hepatitis B virus infection: An insight into infection outcomes and recent treatment options. Virusdisease. 2015;26(1-2):1-8.
5. Hoofnagle JH. Reactivation of hepatitis B. Hepatology. 2009;49(S5):S156-S65.
6. Smalls DJ, Kiger RE, Norris LB, Bennett CL, Love BL. Hepatitis B Virus Reactivation: Risk Factors and Current Management Strategies. Pharmacotherapy. 2019;39(12):1190-203.
7. Wang D, Lv W, Zhang S, Zhang J. Advances in the Research on Anticardiolipin Antibody. J Immunol Res. 2019;2019:8380214.
8. Hossri S, Shadi M, Hamarsha Z, Schneider R, El-Sayegh D. Clinically significant anticardiolipin antibodies associated with COVID-19. J Crit Care. 2020;59:32-4.
9. Ambrosino P, Lupoli R, Tarantino P, Di Minno A, Tarantino L, Di Minno MND. Viral hepatitis and anti-phospholipid antibodies positivity: A systematic review and meta-analysis. Digestive and Liver Disease. 2015;47(6):478-87.
10. Abdel-Wahab N, Talathi S, Lopez-Olivo MA, Suarez-Almazor ME. Risk of developing antiphospholipid antibodies following viral infection: a systematic review and meta-analysis. Lupus. 2018;27(4):572-83.
11. Habibagahi Z, Nazarinia MA, Aflaki E, Rajaee A. Anticardiolipin and antibeta2glycoproteinI antibodies in patients with hepatitis B and C infections. Iran J Immunol. 2007;4(3):161-6.
12. Elefsiniotis IS, Diamantis ID, Dourakis SP, Kafiri G, Pantazis K, Mavrogiannis C. Anticardiolipin antibodies in chronic hepatitis B and chronic hepatitis D infection, and hepatitis B-related hepatocellular carcinoma. Relationship with portal vein thrombosis. European Journal of Gastroenterology & Hepatology. 2003;15(7):721-6.
13. Guglielmone H. Cofactor dependence and isotype distribution of anticardiolipin antibodies in viral infections. Annals of the Rheumatic Diseases. 2001;60(5):500-4.
14. Zachou K, Liaskos C, Christodoulou DK, Kardasi M, Papadamou G, Gatselis N, et al. Anti-cardiolipin antibodies in patients with chronic viral hepatitis are independent of beta2-glycoprotein I cofactor or features of antiphospholipid syndrome. Eur J Clin Invest. 2003;33(2):161-8.
15. Mendoza-Pinto C, García-Carrasco M, Cervera R. Role of Infectious Diseases in the Antiphospholipid Syndrome (Including Its Catastrophic Variant). Current Rheumatology Reports. 2018;20(10):62.

16. Huh JY, Yi DY, Hwang SG, Choi JJ, Kang MS. Characterization of antiphospholipid antibodies in chronic hepatitis B infection. Korean J Hematol. 2011;46(1):36-40.

17. Gharavi AE, Pierangeli SS, Harris EN. New developments in viral peptides and APL induction. J Autoimmun. 2000;15(2):227-30.

18. Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. Semin Arthritis Rheum. 2002;31(4):256-63.

19. Jizzini M, Shah M, Zhou K. SARS-CoV-2 and Anti-Cardiolipin Antibodies. Clin Med Insights Case Rep. 2020;13:1179547620980381.

20. Truglia S, Capozzi A, Mancuso S, Manganelli V, Rapino L, Riitano G, et al. Relationship Between Gender Differences and Clinical Outcome in Patients With the Antiphospholipid Syndrome. Frontiers in Immunology. 2022;13.

21. de Carvalho JF. Influence of gender on the clinical and laboratory spectra of patients with primary antiphospholipid syndrome. Rheumatol Int. 2011;31(5):647-50.

22. Galli L, Gerdes VE, Guasti L, Squizzato A. Thrombosis Associated with Viral Hepatitis. J Clin Transl Hepatol. 2014;2(4):234-9.

23. Devreese KMJ, Zuily S, Meroni PL. Role of antiphospholipid antibodies in the diagnosis of antiphospholipid syndrome. J Transl Autoimmun. 2021;4:100134.