Association of Anti-Phospholipid Antibodies and Hepatitis B Virus Infection in Najaf Governorate

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Abstract

Background: Antiphospholipid syndrome (APS) is an autoimmune disease with multifactorial risk factors including genetic and environmental factors such as viral infection, and it can be diagnosed by the presence of antiphospholipid antibodies (APLA) in the serum. Hepatitis B virus (HBV) is one of the most prevalent viral infections in the world and has been connected to a number of autoimmune illnesses. Some studies suggested that HBV might be the cause of APLA production and APS development. No previous study was done in Iraq regarding the relationship between these two. **Objective:** To estimate the prevalence of APLA in HBV-infected individuals in Najaf. **Materials and Methods:** A cross-sectional study was done in Najaf from September 2022 to March 2023, and it included 113 patients (70 were males, age range was 15–85 years) with HBV infection and without any history of APS. They were tested for Hepatitis B core (HBc) total and immunoglobulin M (IgM) Abs and then tested for APLA by enzyme-linked immunosorbent assay (ELISA) technique. The Statistical Program for Social Sciences (SPSS) version 26.0 was used for statistical analysis. **Results:** Only four patients were positive for HBc total and IgM the remaining 109 were positive for IgG only, 38 (33.63%) were positive for APLA, and the age group (41–50 years) with the highest rate of APLA positivity. Non-significant statistical difference was seen between APLA and gender or age. Acute HBV had higher titers of APLA. **Conclusion:** HBV may lead to the production of APLA in some patients without any other risk factors, especially in middle-aged patients.

Keywords: Antiphospholipid antibodies, hepatitis B virus, Najaf

Introduction

Hepatitis B virus (HBV) is a hepatotropic virus that can cause a chronic infection due to immunological anergy in humans.[1]

HBV is a huge public health issue all over the world. It is still the leading cause of chronic hepatitis. The number of persons who have had HBV for a long period is estimated to be between 350 and 400 million. This represents approximately seven percent of the global population. Nevertheless, an Iraqi study published in 2013 found that the frequency of HBV positive in Iraq is low.[2] Antiphospholipid antibodies (APLA), namely lupus anticoagulant (LA), anti-cardiolipin antibodies (ACLA), and anti-*B*2-glycoprotein type I (B2GPI) antibodies have the potential to cause significant health implications such as antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE), and recurrent miscarriage.[3-5] APS is more likely in the presence of various infections, with a special emphasis on viral infections. The most common infection related to APLA is the hepatitis C virus. These antibodies have been associated with thromboembolic events such as catastrophic APS.[6] The incidence of elevated APLA antibodies and associated thromboembolic events may be heightened by viral infection.[7]

APLA were found in HBV-infected patients, and they showed a modest correlation with clinical signs of APS.[8]

APS coexisting clinical signs include livedo reticularis, cutaneous ulcerations, thrombocytopenia, hemolytic anemia, valvular heart disease, and nephropathy.[7]

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As there has been no previous research on the association between APLA and HBV in Najaf-Iraq, this study focused on the prevalence of these antibodies as well as their distribution among acute and chronic HBV.

Materials and Methods

Study design and patients included

This is a cross-sectional study that was held in the province of Najaf between September 2022 and March 2023, it included 113 HBV-infected patients who attended to The Public Health Laboratory either referred by gastroenterology (GIT) specialist or send from the Main Blood Bank or by routine checking (70 were men), age ranged from 15 to 85 years (median was 43 years).

Sample processes and techniques

Five milliliters of whole blood were collected from each patient into gel tubes, allowed to clot at room temperature. Next, it was separated using a centrifuge at 4000 round per minute (RPM) for 10 min. Then, the serum samples were taken and divided into four Eppendorf tubes, one for liver enzyme glutamate aminotransferase glutamic pyruvic transaminase (GPT) test and the other three were stored in deep freezer (–80ºC) for later use.

Inclusion criteria were being positive for HBs Ag and exclusions criteria were SLE, APS, history of recurrent miscarriages, and infertility with unexplained cause.

All samples included were tested for HBs antigen by diagnostic enzyme-linked immunosorbent assay (ELISA) kit (manufactured by Fortress Company, Antrim, UK) and all were positive then confirmed by double testing. After that, they were tested for both Hepatitis B core (HBc) total antibodies using commercial ELISA kits by Abia, Berlin, Germany and immunoglobulin M (IgM) HBc antibodies by Sun Long Biotech, Hangzhou, China, GPT level was estimated using biochemistry auto-analyzer (NeoChem 100 by Neomedica, Serbia). Lastly, all patients were screened for APLA by quantitative ELISA kit by (Sun Long Biotech); all procedures were performed according to the manufacturer's instructions and all positive results were confirmed by double testing.

Statistical analysis

IBM SPSS version 26 (Chicago, IL, USA) was used for statistical processes. Mean and standard deviation were used for parametric data, the Chi-Square test was used for categorical variables, Mann-Whitney *U* test and Pearson correlation were also employed for non-parametric variables.

Ethical approval

This study was approved by The Ethical Committee of the college of Medicine, University of Kufa.

The approval letter is numbered: MEC-42 and dated May 23, 2023.

Results

Demographic data and glutamic pyruvic transaminase result

One hundred and thirteen patients with positive HBs antigen were included; all of them accepted to participate in the study by giving a serum sample and information/data, the majority were male patients (62.83%).

The highest rate of infection was with age group 31–40 years, and it was followed by age group 31–40 years. The lowest rate was seen in age group less than 20 years. Their liver enzyme GPT test revealed that only 26.54% of them had high results as shown in Table 1.

Type of hepatitis B virus infection depending on HBc

By testing their serum for both types of HBc antibodies (IgM and total) the results showed that only four patients were positive for both markers and considered acutely infected, and the remaining 109 were considered chronically infected,[9] as can be demonstrated in Table 2.

Antiphospholipid antibodies

The levels of APLA were divided into four groups depending on the titers (1-titers less than 15 were considered negative for APLA, 2-titers between 15 and 20 were considered weak positive, 3-titers between 20 and 40 were considered moderately positive, 4-titers equal to or higher than 40 were considered strong positive) as can be seen in Table 3. The prevalence of APLA among

Table 1: Distribution of HBV-positive patients by age, gender, and GPT test findings

	Number	%
Gender groups		
Male	71	62.83
Female	42	37.17
Age groups		
<20 years	9	8
20–30 years	17	15
31–40 years	25	22.12
41–50 years	31	27.43
51–60 years	10	8.84
61–70 years	10	8.84
>70 years	11	9.73
GPT (U/mL)		
Normal (<40)	83	73.45
Abnormal (>40)	30	26.55

Table 2: Type of HBV infection distribution

Type of HBV infection	Number	%
Acute (+ve for both IgM and IgG HBc antibodies)	4	3.54
Chronic (+ve for only IgG HBc antibodies)	109	96.46

HBc: Hepatitis B core, IgG: immunoglobulin G, IgM: immunoglobulin M

Table 3: Distribution of antiphospholipid antibodies among HBV infected patients

APLA result	Number	%	
Negative (<15 IU/mL)	75	66.37	M = 10
A weak positive (15–20 U/mL)	15	13.27	IQR = 16.02
Moderately positive (20–40 U/mL)	20	17.7	
High positive (>40 U/mL)	3	2.65	

M: median, IQR: interquartile range

Table 4: The distribution of APLA according to demographic data

	Antiphospholipid antibodies result				
	Negative	Low positive	Moderate positive	High positive	
Gender					$X^2 = 0.797$, $P = 0.850$
Male	49	9	11	2	
Female	26	6	9	1	
Age groups					
<20 years	7	0	2	0	$X^2 = 24.17$, $P = 0.149$
20–30 years	11	3	2	1	
31–40 years	17	2	6	0	
41–50 years	20	2	7	2	
51–60 years	8	1	1	0	
61–70 years	3	5	2	0	
>70 years	9	2	0	0	
GPT test result					
<40 U/mL	55	13	14	1	$X^2 = 3.94$, $P = 0.268$
>40 U/mL	20	2	6	2	

P: probability of chance (level of significance < 0.05), X^2: Chi square test

Table 5: Pearson correlation between APLA and age, GPT

		Age	GPT
Antiphospholipids Antibody	Pearson correlation	–0.059	–0.089
	P	0.535	0.347

Table 6: Mann–Whinny *U* test between APLA and types of HBV infection

Acute	*M* = 97.38 *N* = 4	Mann-Whitney *U* = 56.500	**P = 0.12**
Chronic	*M* = 55.52 *N* = 109		

M: mean ranks, N: number of cases, *P*: probability level (level of significance < 0.05), Bold value indicates no significant difference between acute and chronic HBV infection regarding the production of anti phospholipid antibodies

HBV-positive patients in this study was (33.63%). Most of these positive cases had moderate titers (20–40 U/mL). The median and (interquartile range) was 10 (16.02).

Distribution of antiphospholipid antibodies according to demographic data and glutamic pyruvic transaminase

Non-significant association was seen between APLA and gender, age groups, and GPT results, as seen in Table 4.

There was a non-significant negative relationship between APLA and both Age and GPT level [Table 5].

Acutely infected (positive for both total and IgM HBc antibodies) had higher results for APLA than those with chronic HBV, as seen in Table 6.

Discussion

In Table 1, male patients were higher in rate of HBV infection than female patients (62.83% vs. 37.17%); the ratio is (1.69:1). This agrees with a Pakistani Study that reported male patients were higher in chronic HBV infection rate compared to female (2.14:1).[10] An Iranian Meta Analysis conducted in 2016 revealed than the rate of male patients was higher than female patients.[11] The reason could be due to estrogen hormone that protect the hepatocyte against the development of chronic HBV as reported by Baig.[12]

The highest infection rate was seen in the age groups (41–50 years) with 27.43% of the total study population, which is different from what was reported by other studies.[10]

Antiphospholipid antibodies and thromboembolic manifestations are associated viral hepatitis.[13]

The APLA antibodies kit used in the present study is manufactured by Sunlong Biotech Co., Ltd and used to detect the presence of all three types of Antiphospholipids antibodies LA, ACLA, and B2GPI, without determining the specific type, because LA requires plasma sample in sodium citrate tube and our samples are serum only; thus

LA is excluded, and only ACLA and B2GPI are possible to be found.

Previous studies reported that APLA prevalence among HBV infected patients were varied between 12% and 42%[8,14,15] this variance is due to different methodological methods or due variable cut-off values.

In Iraq, one study by Iqbal in 2020 reported that ACLA which is one type of APLA, It presence was 14%[16] but their exclusion criteria were different than the criteria mentioned in the current study. In this study, 33.63% of HBV-infected individuals had APLA.

As seen in Table 3 the antiphospholipid antibodies results are divided into four categories to determine the level of severity. Most of the positive subjects had moderate levels of APLA (20–40 U/mL), yet the presence of high positive APL (>40) was seen only in 2.65% of HBV infected subjects, according to a study by Katrien Devreese in 2010 reported that only subjects with (99th percentile or >40 IgG phospholipid units [GPL] or IgM phospholipid units [MPL]) are associated with APS manifestations.[17]

The highest rate for positive APLA was seen within the age group (41–50 years). This is in agreement with Huh,[8] who reported the highest number of cases were within the same age group.

In Table 6, there was negative non-significant correlation between APLA and GPT and age. This means that higher levels of APLA titer are seen in young age patients with lower GPT levels. This might be due to a stronger immune system possessed by younger patients which continuously produces APLA, and also the ability of the immune system to restore normal liver function.

Regarding Table 5, Mann–Whitney *U* test results showed that the type of HBV infection was significantly associated with APLA production. The subjects within the acute group had higher APLA titers than those of the chronic type. This was not reported previously up to our knowledge.

The postulated mechanism for the generation of aPLs in viral infections is molecular mimicry. Previous studies demonstrated that antigenic determinants shared by infectious agent antigens and host tissue may trigger the immune response.[18,19] However, the precise process that can lead to the production of aPLs and their potential pathophysiological ramifications in infected patients are not yet well understood.

Whether these antibodies are transient or persistent cannot be determined and follow-up prospective studies are needed.

Conclusion

1 Anti phospholipid antibodies occur in some patients with HBV infection.
2 Not only chronic HBV leads to the production of APLA but also acute HBV may do exactly the same thing.
3 Younger HBV infected patients had higher titers than older ones.
4 Most of the positive APLA had low-moderate titers and only few had high positive (>40).

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Conflict of interest

There is no conflict of interest.

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