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# ANTIPHOSPHLIPIDS ANTIBODIES IN CHRONIC HEPATITIS B PATIENTS PRESENTING AT A TERTIARY CARE HOSPITAL OF SINDH

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## ABSTRACT

**Objective:** To investigate the antiphospholipid antibodies in chronic hepatitis B patients presenting at a tertiary care hospital of Sindh. **Study design:** Case control study. **Place and Duration:** Department of Medicine, Liaquat University of Medical and Health Sciences Jamshoro from January 2016 to December 2016. **Subjects and Methods:** The present study included 100 chronic hepatitis B and 100 controls. Anticardiolipin antibodies IgG, IgM and β2-glycoprotein I IgG and lupus anticoagulant (LA) were detected. Data was analyzed on SPSS 22.0 (IBM, Incorporation, and USA) at 95% confidence interval ($P \leq 0.05$). **Results:** Anticardiolipin IgG, anticardiolipin IgM, and $\beta_2$-Glycoprotein I IgG were noted as 3 and 19%, 2% and 15%, 0% and 37%, & 1% and 7% respectively. Anticardiolipin IgG, IgM, and $\beta_2$-Glycoprotein I IgG were noted as 10.74±1.61 and 13.80±3.5 U/ml, 11.12±1.50 and 15.32±3.62 U/ml & 132.7±.19 and 206.3±16.8 µg/ml in control and cases respectively ($P<0.05$). Anticardiolipin IgG showed positive correlation with serum creatinine, PT, APTT, anticardiolipin IgM and $\beta_2$-Glycoprotein I IgG. **Conclusion:** In conclusion, the present study found antiphospholipid antibodies anticardiolipin IgG, anticardiolipin IgM, $\beta_2$-Glycoprotein I IgG and lupus anticoagulant in the hepatitis B infected.

**KEYWORDS:** Anticardiolipin IgG, Anticardiolipin IgM, β2-glycoprotein I IgG, Lupus anticoagulant, Hepatitis B virus.

## INTRODUCTION

Antiphospholipid antibodies (aPLs) are heterogeneous autoantibodies directed against the phospholipids binding proteins.[1] The aPLs consist of anticardiolipin IgG, anticardiolipin IgM and $\beta_2$-Glycoprotein I IgG and the Lupus anticoagulant. They are inducer of different autoimmune diseases.[2] The aPLs are raised in the antiphospholipid syndrome (APS) which may be primary APS and or secondary APS. The APS is characterized by the clinical manifestations of hyper coagulation, thrombocytopenia, thrombosis and embolic phenomena, etc. The aPLs are common cause of recurrent spontaneous abortions.[3] The aPLs have affinity for the anionic phospholipids.[4] Presence of aPLs is an established risk factor for vascular thrombosis and embolism with clinical manifestations depending on the body part.[5] Clinical manifestations include thrombocytopenia, bleeding tendency, recurrent arterial thrombi, recurrent venous thrombi, neurological deficits as transient ischemic attack (TIA) and brain stroke, skin disease as livedo reticularis. Pathogenesis of aPLs mediated injury occurs through activation of coagulation and complement cascade, endothelial dysfunction and platelet activation.[6] The aPLs have also been found in patients suffering from the infectious diseases, like the streptococci, staphylococci, H pylori, Parvovirus B19, Herpes zoster, human immunodeficiency virus (HIV), and chronic viral hepatitis. The hepatitis B virus (HBV) and hepatitis C virus (HCV), prevalent in Pakistan, are also characterized by the presence of aPLs.[7] Reported prevalence of aPLs shows major variations across studies.[8,9] Variations of prevalence have been attributed to the differences of aPLs detection, type of aPLs antibody, infections, etc. For example patients of HIV showed high prevalence of 46.5% of anti cardiolipin antibodies[10] and 43% of lupus anticoagulant.[11] While the β2-glycoprotein I antibodies had rarely been detected in the HIV patients.[11] Clinical significance of aPLs in infections is highly controversial. The presence of aPLs in infections has been regarded as non-pathogenic as reported.[11,12] While other studies[13,14] have reported infection related aPLs are risk for the thrombosis, such as the pulmonary embolism or portal vein thrombosis, etc. Several studies have reported high prevalence of anticardiolipin antibodies in chronic viral hepatitis compared to controls.[15,16] Currently the viral hepatitis

both hepatitis B and C are on rise in Pakistan and research is lacking on the aPLs in these patients. The concomitant presence of aPLs in chronic hepatitis B infection might be increasing the morbidity and mortality in this population group. Hence there is need of conducting more research investigations on the presence of aPLs in chronic hepatitis B viral infection. The present research investigated the antiphospholipid antibodies in chronic hepatitis B patients presenting at a tertiary care hospital of Sindh.

## SUBJECTS AND METHODS

A Case-control study was conducted at **Department of Medicine, Liaquat University of Medical & Health sciences hospital Hyderabad from January 2016 to December 2016**. A sample of 100 cases (chronic active hepatitis B) and 100 controls (healthy subjects) was selected according to inclusion and exclusion criteria. Inclusion criteria for the chronic hepatitis B patients were; positive HBeAg, and HBV-DNA ≥104 copies/mL, alanine aminotransferase (ALT) > 3 times of upper normal limit and age >40 years. Inclusion criteria for controls were; Hepatitis B negative, anti- HCV negative, anti-HIV negative, and antinuclear antibody negative, and age >40 years. Chronic hepatitis B patients with negative HBeAg and HBV-DNA <104 copies/mL) and taking anti viral therapy were excluded.[17] ALT levels of 5-40 IU/L was taken as normal. Informed consent was signed by all volunteer participants both controls and patients. Volunteers were taken into confidence through interviews of their benefits and loss. They were informed that the present research will help the Hepatitis B patients in future for additional problems which they might be facing. 10 ml of venous blood was collected in a disposable syringe (BD, USA) from the prominent vein in particular located in the cubital fossa. The blood was centrifuged for separate the sear at 4000 rpm for 15 minutes. The aPLs i.e. the anticardiolipin IgG and anticardiolipin IgM were measured from patients and control sera. By the ELISA assay kit (Abcam, USA). The aPLs- $\beta_2$-Glycoprotein I IgG was also detected by the ELISA assay kit (Abcam, USA). Normal range of anticardiolipin IgG and anticardiolipin IgM was taken as between 5 and 15 U/ml. Levels of >15 U/ml for anticardiolipin IgG and anticardiolipin IgM were taken abnormal. While the reference range of aPLs- $\beta_2$-Glycoprotein I IgG of <200 µg /mL and ><200 µg /mL as abnormal. LA was detected by dilute Russell's viper venom test (HemosIL Kit, Instrumentation Laboratory, Milano, Italy). LA was reported as positive or negative according to the Subcommittee on Lupus Anticoagulant/Phospholipid Dependent Antibodies Guidelines.[15] A structured proforma was used for data collection including biodata. The study was conducted according to the "declaration of Helsinki" and approved by the Ethics Committee of our institute. Informed written consents proforma signing was mandatory for volunteer participation in the study. Statistical software SPSS 22.0 (IBM, Incorporation, USA) was employed for the research variables analysis. Chi square test analysed the categorical variables such as gender and lupus anticoagulant. Student`s t-test analyzed the continuous variables such as the age, blood pressure, anticardiolipin antibodies, etc. Pearson`s correlation was employed for the association of variables among each other. 95% confidence interval was taken as statistically significant ($P < 0.05$).

## RESULTS

Of 200 control and cases, the male subjects predominated. Male and female in control and cases were found as 78% and 22%, 81% and 19% respectively (P<0.05). Majority of study subjects were in their fifth decade of age. The mean ±SD age was 49.54± 4.54 and 50.35± 5.10 years in controls and cases respectively (P=0.09) (table 1). Biochemical tests are shown in the table 1. Serum creatinine, Prothrombin time, APTT, ALT, Platelet counts, anticardiolipin IgG, anticardiolipin IgM, and $\beta_2$-Glycoprotein I IgG revealed significant differences between controls and cases (table 1). Anticardiolipin IgG, anticardiolipin IgM and $\beta_2$-Glycoprotein I IgG were found as 10.74±1.61 and 13.80±3.5 U/ml, 11.12±1.50 and 15.32±3.62 U/ml & 132.7±.19 and 206.3±16.8 µg/ml in control and cases respectively (P<0.05). Frequency of anticardiolipin IgG, anticardiolipin IgM, and $\beta_2$-Glycoprotein I IgG were noted as 3 and 19%, 2% and 15%, 0% and 37%, & 1% and 7% respectively (P<0.05) (table 2). Anticardiolipin Ig G shows positive correlation with serum creatinine (r= 0.44, P=0.0001), PT (r= 0.334, P=0.0001), APTT (r= 0.242, P=0.0001), anticardiolipin IgM (r= 0.53, P=0.0001) and $\beta_2$-Glycoprotein I IgG (r= 0.591, P=0.0001). Platelet counts were negatively correlated with anticardiolipin IgG (r= -0.285, P=0.0001) (table 3, Graphs 1- 6).

**Table 1. Characteristics and laboratory findings of study subjects**

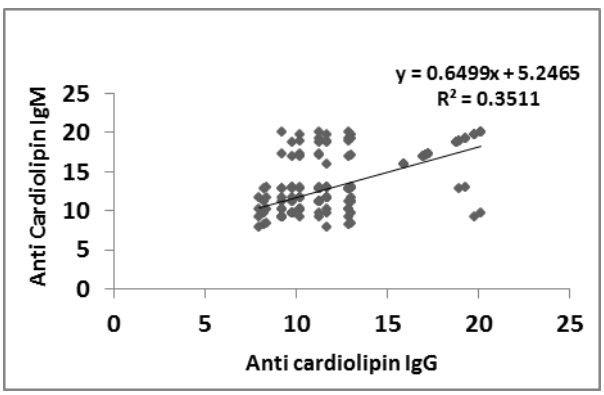
| | Control | | Cases | | P- value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Age (years) | 49.54 | 4.54 | 50.35 | 5.10 | 0.09 |
| Body weight (kg) | 70.30 | 6.02 | 70.92 | 5.27 | 0.90 |
| Systolic BP (mmHg) | 132.32 | 9.64 | 132.2 | 9.51 | 0.99 |
| Diastolic BP (mmHg) | 69.40 | 6.01 | 69.35 | 5.71 | 0.95 |
| Blood glucose (R) (mg/dl) | 131.96 | 8.70 | 132.0 | 12.0 | 0.97 |
| HbA1c (%) | 5.48 | 0.88 | 5.34 | 0.90 | 0.98 |
| S. Creatinine (mg/dl) | 0.91 | 0.20 | 1.53 | 0.43 | 0.0001 |
| S. Cholesterol (mg/dl) | 151.2 | 29.6 | 158.1 | 30.75 | 0.10 |
| Alanine transaminase (IU/L) | 23.8 | 11.90 | 60.52 | 19.52 | 0.0001 |
| Prothrombin time (sec) | 9.89 | 1.90 | 13.52 | 1.02 | 0.0001 |
| APTT (sec) | 27.21 | 4.47 | 30.13 | 4.83 | 0.0001 |
| Platelet counts ($x/10^9$) | 4.68 | 0.83 | 3.32 | 1.40 | 0.0001 |
| AntiCL-IgG (U/ml) | 10.74 | 1.61 | 13.80 | 3.59 | 0.0001 |
| AntiCL-IgM (U/ml) | 11.12 | 1.50 | 15.32 | 3.62 | 0.0001 |
| $\beta_2$-Gp I IgG (µg /mL) | 132.72 | 9.19 | 206.3 | 16.8 | 0.0001 |

**Table 2. Frequency of antiphospholipid antibodies**

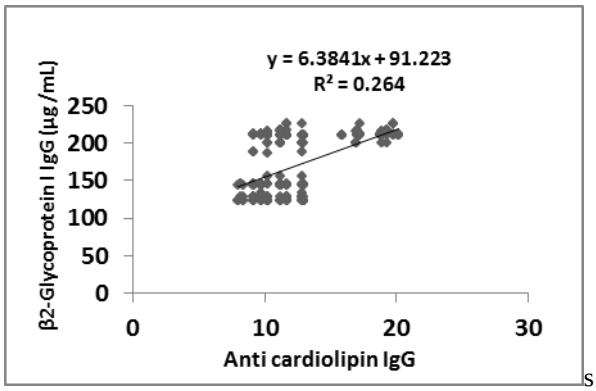
| | Control | Case | P- value |
|---|---|---|---|
| Anticardiolipin IgG (U/ml) | 3% | 19% | 0.0001 |
| Anticardiolipin IgM (U/ml) | 2% | 15% | 0.0001 |
| $\beta_2$-Gplycoprotein I IgG (µg /mL) | 0% | 37% | 0.0001 |
| Lupus anticoagulant | 1% | 7% | 0.0001 |

**Table 3. Pearson`s correlation of Anticardiolipin IgG**

| | r-value | P-value |
|---|---|---|
| S. creatinine | 0.494** | 0.0001 |
| Prothrombin time | 0.334** | 0.0001 |
| APTT | 0.242** | 0.0013 |
| Platelet count | -0.285** | 0.0012 |
| AntiCL-IgM (U/ml) | 0.593** | 0.0001 |
| β2-Gp I IgG (µg /mL) | 0.591** | 0.0001 |
| **. Correlation is significant at the 0.01 | | |




**Graph 1. Scatter plot showing positive correlation of anticardiolipin IgG and IgM**

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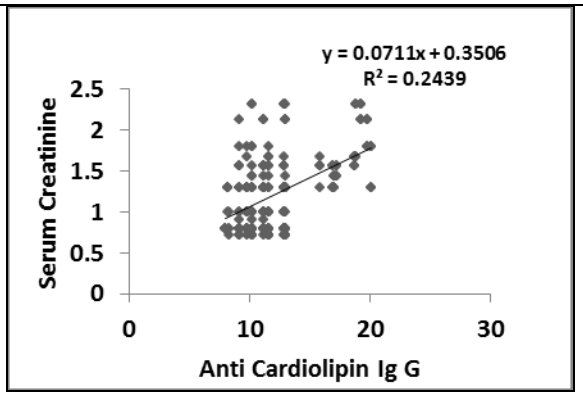
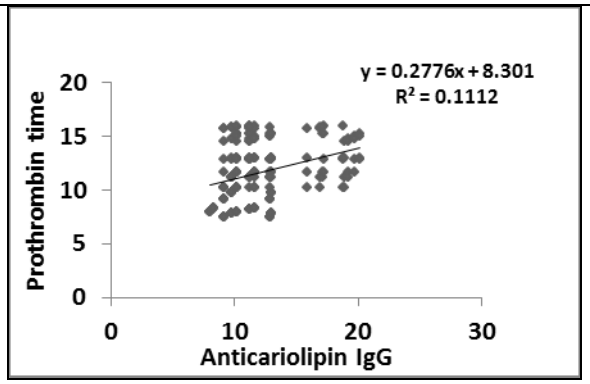
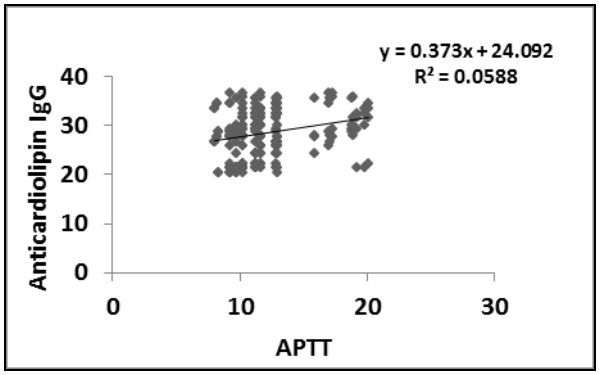
**Graph 2. Scatter plot showing positive correlation of anticardiolipin IgG and β2-Gp I IgG**



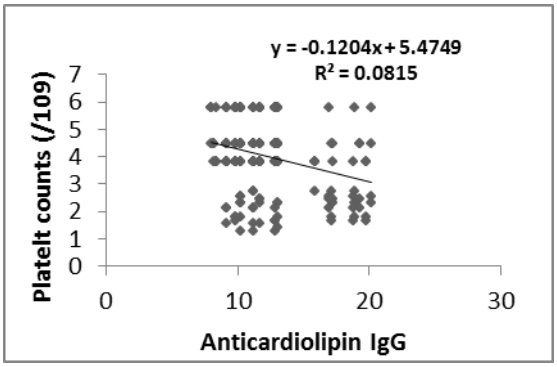

**Graph 3. Scatter plot showing positive correlation of anticardiolipin IgG and serum Creatinine**




**Graph 4. Scatter plot showing positive correlation of anticardiolipin IgG and Prothrombin time**




**Graph 5. Scatter plot showing positive correlation of anticardiolipin IgG and APTT**




**Graph 6. Scatter plot showing negative correlation of anticardiolipin IgG and Platelet counts**

## DISCUSSION

The present case control study was conducted to analyze the frequency of antiphospholipid antibodies in the chronic hepatitis B (CHB) cases at our tertiary care hospital. The antiphospholipid antibodies (aPLs) are reported in the autoimmune disorders like the primary biliary cirrhosis, systemic lupus erythematosus, rheumatoid arthritis, etc. The aPLs have also been found in the infectious disease like bacterial disease, viral disease, etc.[9,17] Induction of aPLs is proposed by the molecular mimicry mechanism, particularly in infections cases. Previous studies reported antigenic mimicry of infectious agent's triggers the immune response.[10,11] However, how the aPLs production does occur in infectious disease is not well established. Prevalence of aPLs in the infections varies because of different assay methods. The ideal standard method of aPLs detection is debatable till the day.[12] Discrepancy exists due to the various assay kits, assay methodologies, cut off values, ethnicity and geographical areas. In present study, the serum creatinine, prothrombin time, APTT, platelet counts, anticardiolipin IgG, anticardiolipin IgM and $\beta_2$-Glycoprotein I IgG revealed significant differences between controls and cases ($P<0.05$). Anticardiolipin IgG, anticardiolipin IgM and $\beta_2$-Glycoprotein I IgG were found as 10.74±1.61 and 13.80±3.5 U/ml, 11.12±1.50 and 15.32±3.62 U/ml & 132.7±.19 and 206.3±16.8 µg/ml in control and cases respectively ($P<0.05$). The previous studies have reported prevalence of 14% and 42% for anticardiolipin and 2% and 7.5% for the β2GPI IgG.[13,16,18] The findings are in agreement with the present research. In present study, the frequency of anticardiolipin IgG, anticardiolipin IgM and $\beta_2$-Glycoprotein I IgG were noted as 3 and 19%, 2% and 15%, 0% and 37%, & 1% and 7% respectively ($P<0.05$) (table 2). High frequency of aPLs of present study is supported by previous studies.[13,18] However, Hu et al[17] reported low prevalence; reason was the high cut off value of 20 GPL or MPL as the cut-off values. While present study used 10 GPL (or MPL) cut-off value similar to previous studies.[13,18] In present study, the $\beta_2$-Glycoprotein I IgG was noted 0% and 37% of control and case, similar to those in previous reports.[13,18-20] In present study, the high frequency of $\beta_2$-Glycoprotein I IgG of 37% in case is speculated to be due to the active hepatitis B infection in our patients. It is speculated that the active viral replication exposes the immune cell continuously to antigenic stimulation which results in persistent aPLs genesis. A previous study reported aPLs disappeared after HCV infection eradication, but it soon appeared with relapse of hepatitis C.[21] Hence, it is concluded that the aPLs exists in active infections persistently. Presence of anticardiolipin IgM is least often manifests clinical than the anticardiolipin IgG.[22,23] However, in the present study, the anticardiolipin Ig G was found higher than IgM but the cases were having no manifestations of antiphospholipid syndrome. These findings support the fact that the infection-associated

aPLs rarely manifest clinically.[17] In the present study, the anticardiolipin IgG shows positive correlation with serum creatinine (r= 0.44, P=0.0001), PT (r= 0.334, P=0.0001), APTT (r= 0.242, P=0.0001), anticardiolipin IgM (r= 0.53, P=0.0001) and $\beta_2$-Glycoprotein I IgG (r= 0.591, P=0.0001). While the platelet counts were negatively correlated with anticardiolipin IgG (r= -0.285, P=0.0001). The correlation is being reported for the first time. In conclusion, the chronic hepatitis B active infection shows antiphospholipid antibodies without manifest clinically. Most frequent antibody detected was the $\beta_2$-Glycoprotein I IgG followed by anticardiolipin IgG in the present study. The present study has limitations of small sample size, viral markers were not detected fully, specific ethnicity, different geographical area, and genetic factors. However, the antiphospholipid antibodies may produce extra manifestations hepatitis B patients which should be interpreted and treated accordingly.

## CONCLUSION

In conclusion, the present study reports presence of antiphospholipid antibodies in the hepatitis B infected subjects. Most frequent antiphospholipid antibody detected was the $\beta_2$-Glycoprotein I IgG followed by anticardiolipin IgG and anticardiolipin IgM.

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