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Anticardioliptin Antibodies as a marker of hepatitis C Virus Severity

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ABSTRACT

Background Anticardiolipin antibody (aCL Ab) is considered one of the contributory factors in the development of acute ischemic stroke. Chronic hepatitis C virus infection (HCV) and the antiphospholipid syndrome are two conditions that have increased the risk of stroke. The aim of this study is investigate the prevalence of anticardiolipin autoantibodies IgM (ACA IgM), in serum samples of patients with chronic HCV infection and their relationship to the severity of the viral infection. The study was performed on 75 Egyptian subjects, 25 healthy volunteers and 50 patients of both sexes; all patients were HCV-4a. And divided into two groups according to their real time (RT) PCR into, 25 patients with low viremia, (HCV-RNA) less than 10^6 lU/ml and 25 patients with high viremia (HCV-RNA) more than 10^6 lU/ml. All subjects after fasting for twelve hours and stored for the determination of aminotransferases (ALT, AST) and γ-GT enzymes activities, and anticardiolipin autoantibodies IgM. There is very highly significant increase of serum ACA (IgM) MPL (U/ml) in patients with high viremia and low viremia when compared to the control group ($p < 0.0001$ for both) and high significant increase in patients with high viremia when compared with low viremia ($p < 0.0001$). Also there is high significant correlation between serum ACA (IgM) and quantity of HCV-RNA in both high and low viremia patients. But no significant correlation between serum ACA (IgM) serum ACA (IgM) and serum liver enzymes.

INTRODUCTION

Hepatitis C virus (HCV) infects over 3% of the world population and is the leading cause of chronic liver disease worldwide. Chronic hepatitis C virus (HCV) has been linked to extra hepatic autoimmune phenomena. In addition, a variety of autoantibodies are found in patients with HCV **(1).** Sedimentation analysis and characterization of HCV RNA-

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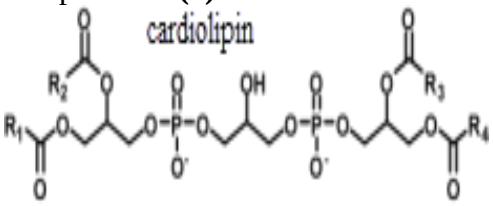
containing particles produced in the cultured cells revealed that HCV virions cover a wide range of heterogeneous densities in sucrose gradient. The fractions of low densities are infectious, while the higher-density fractions containing the majority of HCV virion RNA are not. HCV core protein and Apo lipoprotein B were detected in the infectious HCV virions **(2).** The majority of infectious hepatitis C particles are present in the low-density fractions from plasma of infected patients **(3).**

Cardiolipin is an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition**. (4)**



Anticardiolipin antibody (ACA) is considered one of the contributory factors in the development of acute ischemic stroke. Chronic hepatitis C virus infection (HCV) and the antiphospholipid syndrome are two conditions that have increased the risk of stroke.

Anticardiolipin antibody (ACA) is considered one of the contributory factors in the development of acute ischemic stroke. Chronic hepatitis C virus infection (HCV) and the antiphospholipid syndrome are two conditions that have increased the risk of stroke. Various infectious diseases can induce anticardiolipin antibodies (ACA); however, these antibodies are not usually associated with thrombotic events, as happens with autoimmune diseases, in which these antibodies need the presence of β_2-glycoprotein I (**5),**

Abaci et al (2010) *(6)* reported a high prevalence of IgG and IgM aCL in the serum of patients with HCV infectious diseases. A positive factor for aCL was determined by age, sex, and the severity of HCV infection.

Sjöwall et al (2012) *(7)* reported an increased prevalence of anti-C-reactive protein (CRP) and aCL antibodies in HCV-infected patients. The presence of anti-CRP antibodies was correlated with the presence of rheumatoid factor (RF), cryoslobulinemia, and severity of liver disease however, ACA IgM showed high correlation with the severity of liver disease.

PATIENTS AND METHODS:

The study was performed on 75 subjects, 25 healthy volunteers of both sexes without history of any autoimmune or viral diseases, their age ranged from 40 to 45 years, served as control group. 50 patients from Upper Egypt (Qena, Sohag and Assiut) hospitals matched for age and sex. Written consent was taken from all participants and the results were explained to them. Patients with chronic HCV infection had no evidence of previous hepatitis B virus (HBV) infection or any other autoimmune disorder. All patients were HCV-4a. HCV-RNA was examined for real time PCR (RT-PCR). No evidence of hepatic lesions was detected by ultrasound. Patients were divided into two groups according to their quantitative count by real time (RT) PCR: (group I) low viremia (HCV-RNA) less than 10^6 lU/ml and (group II) high viremia (HCV-RNA) more than 10^6 lU/ml. Serum samples were obtained from all subjects after fasting for twelve hours and stored for the determination of aminotransferases (ALT, AST) and γ-GT enzymes activities, and anticardiolipin autoantibodies IgM

Biochemical Parameters

All patients were already have real time PCR (RT-PCR) results. Serum aminotransferases (ALT and AST) and □-GT were determined according to **Wilkinson et al (1972)** *(8) and* **Szasz (1976)** *(9)* respectively, using commercial kits (Randox, Grumlin, Go. Antirim, UK). Anticardiolipin autoantibodies (ACA)IgM were determined by a sensitive ELISA, as described by **Emlen (1996)***(10)* using kit purchased from INOVA Diagnostics, Inc. (USA).

Principles of the Procedure of (ACA) IgM:

Purified cardiolipin antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any cardiolipin antibodies present to

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bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgM conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgM to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgM, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. After stopping the enzymatic production of colored product, the presence or absence of cardiolipin antibody is determined by comparing the sample optical density with that of a five point calibration curve. Results are reported out semi-quantitatively in standard IgM anticardiolipin units (MPL).

Calculation of Results

1) We determine the mean value for all duplicate readings.
2) We plot the log of mean absorbance of the Calibrator curve for the ACA IgM III assay against the log of their concentrations.as in fig.(1)
3) Then we determine the unknown ACA MPL concentration from the "X" axis by reading the corresponding absorbance on the "Y" axis.

STATISTICAL ANALYSES: were conducted by using the GraphPad Prism 5. Results were expressed as means ± SD & SEM. Comparisons between groups were made using Student's unpaired t -test, simple (Pearson) correlation coefficients between different variables were calculated. Probability levels less than 0.05 were considered significant.

RESULTS : Table (1)& fig.(2) shows comparison between HCV- RNA concentration of patients with low and high viremia which was found to be 470900.0000 ± 268912.39796 and 6670000.0000 ± 2830410.25687 IU/ml respectively (p value <0.0001). Table (2) & fig (3) shows very highly significant increase of serum ACA(IgM) MPL(U*/ml) in* patients with high viremia and low viremia when compared to the control group ($p < 0.0001$ for both) and high significant increase in patients with high viremia when compared with low viremia ($p < 0.0001$) .Tables (3,4,5) & figures (4,5,6) depicts serum ALT, AST, and γ -GT activities of patients with low and high viremia. AST and ALT showed very high significant increase in high viremia patients ($p<0.0001$ for both) and in low viremia ($P <0.0001$ for AST & <0.00025 for ALT) when compared with controls .But γ-GT shows very high significant increase in high viremia ($p< 0.0001$) and high significant increase in low viremia ($p=0.0001$) when compared with controls. Comparing enzymes of high viremia with low viremia revealed very high significant increase in ALT($p=0.0001$), very high significant increase in AST ($p=0.0001$) and significant increase in γ-GT $p=0.0159$).There is very high significant positive correlation between serum level of anticardiolipin antibodies IgM and HCV-RNA concentration in high viremia patients ($r= 0.9118, p<0.0001$) Table (6) and fig (7)and in low viremia patients ($r=0.766$, $p<0.0001$) table(7) and fig (8). Non-significant correlation between serum levels of anticardiolipin antibodies' and ALT serum levels in high viremia patients table (8).

Results of the present study clearly showed a high prevalence of ACA-IgM antibodies in the serum of patients with HCV (high viremia) group compared to the control group and low viremia group. Our results are in harmony with those of **(Atluri and Rizwan et al. (2017**) (12), **Huh et al. (2011**) (13) and **Abaci et al (2010)** (14) where they reported high prevalence of IgM ACA in patients with HCV. **Gatselis et al. (2015)** (15), demonstrated a significantly higher prevalence of ACA-IgM in patients with autoimmune hepatitis (AIH) compared to other diseases and healthy people. ACA-IgM in AIH may contribute to the progression of liver disease or antiphospholipid syndrome (APLS) clinical manifestations (thrombosis, pregnancy morbidity, and thrombocytopenia) development. **Brusch (2016)** (16); **(Elsayeh et al 2011)** (17), and **Malsuda et al. (1995)** (18)**:** suggested that ACA-IgM seem to be an epiphenomenon, and they do not have clinical or laboratory significance in HCV patients.

However, **González-Reimers et al. (2016)** (19) found more liver fibrosis in patients with HCV and ACA-IgM. In addition, **Abaci et al (2010)** (20) suggested that anticardiolipin antibodies associated with HCV might be an important marker for acute ischemic stroke. The higher prevalence and titer of ACA-IgM in patients with HCV infection suggests that these humoral factors might be involved m the pathogenesis of ischemic stroke. Our results support the mechanism proposed by **Atluri and Rizwan (2017)** (21) where both suggested that, patients with HCV who showed high ACA-IgM had a higher incidence of viremia together with hyper gammaglobulinemia and anti-nuclear antibodies, suggesting the existence of a chronic inflammatory state. Persistent HCV infection leads to endothelial and hepatic damage that can cause alteration of the expression of cell surface phospholipids and induction of pro inflammatory cytokines, which together may promote the generation of ACA-IgM.

CONCLUSION: ACA IgM has high prevalence in patients with high viremia. Also ACA IgM was highly significantly correlated with HCV infectivity. Thus ACA IgM can be considered a good predictor of high viremia in chronic hepatitis C Egyptian patients

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Table (1): comparison between quantitative count of HCV-RNA in patients with chronic HCV (low viremia < 10^6 and high viremia > 10^6 IU/ml)

Parameter (Mean ± SEM)	Low viremia N=25	High viremia N=25	P value
HCV-RNA Concentration(IU/ml)	470900 ± 85038	6670000.0000 ± 895054	<0.0001 *** VHS

N, number of cases VHS, very high significant

Table (2): Comparison between serum anticardiolipin IgM antibodies PMLU/L in low, high viremia patients and controls

	Control	Group I <1000000 IU/ml Unit	Group II >1000000 IU/ml Unit
Mean	0.2040	3.1640	10.6960
Std. Deviation	± 0.3064	± 3.96639	± 3.15600
N	25	25	25
P value vs controls		<0.0001*** VHS	< 0.0001*** VHS
P value vs low viremia			<0.0001*** Sig.

N, number of cases VHS, very high significant

Table (3): Comparison between serum levels of ALT (U/L) in low, high viremia patients and controls

	Control	Group I <1000000 IU/ml	Group II >1000000 IU/ml
Mean	20.6800	37.8000	99.6800
Std. Deviation	± 5.7425	± 20.70427	± 36.65801
N	25	25	25
P value vs controls		0.0005*** VHS	< 0.0001*** VHS
P value vs low viremia			< 0.0001*** VHS

N, number of cases VHS, very high significant

Table (4): Comparison between serum levels AST(u/L) in low, high viremia patients and controls

	Control	Group I <1000000 IU/ml	Group II >1000000 IU/ml
Mean	18.6800	41.0400	81.2400
Std. Deviation	± 5.5955	± 17.1062	± 28.12366
N	25	25	25
P value vs controls		< 0.0001*** VHS	< 0.0001*** VHS
P value vs low viremia			< 0.0001*** VHS

N, number of cases VHS, very high significant

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Table (5): Comparison between serum levels γ-GT (U/L) in low, high viremia patients and controls

	Control	Group I <1000000 IU/ml	Group II >1000000 IU/ml
Mean	20.1600	47.9200	64.9200
Std. Deviation	± 4.7141	±25.26183	±22.75214
N	25	25	25
P value vs controls		< 0.0001*** VHS	< 0.0001*** VHS
P value vs low viremia			0.0159* Sig.

N, number of cases VHS, very high significant

Table (6): Correlation between quantitative count of HCV-RNA (IU/ml) and ACA IgM (PMLU/ml) in high viremia patients

parameters	Mean ± SEM	Pearson r	P value	Significance	N
HCV-RNA(IU/ml)	6670000.0000 ± 2830410.25687	0.9118	< 0.0001	***VHS	25
ACA IgM MPLU/ml	10.6960± 3.15600				

N= number of cases, VHS= very high significant

Table (7): Correlation quantitative count quantitative count between HCV-RNA (IU/ml) and ACA IgM (PMLU/ml) in low viremia patients

parameters	Mean ± SEM	Pearson r	P value	Significance	N
HCV-RNA(IU/ml)	470900.0000 ± 268912.39796	0.766	< 0.0001	***VHS	25
ACA IgM MPLU/ml	14.300 ± 4.10420				

N= number of cases, VHS= very high significant

Table (8): Correlation between ALT (IU/L) and CAIgM(PMLU/ml) in high viremia patients

parameters	Mean ± SEM	Pearson r	P value	Significance	N
ALT (IU/L)	99.6800± 36.65801	0.1506	0.4724	NS	25
ACA IgM MPLU/ml	10.6960 ± 3.15600				

N= number of cases, VHS= very high significant, NS= non-significant

Fig.1. Anticardiolipin IgM (ACA-IgM) calibration curve

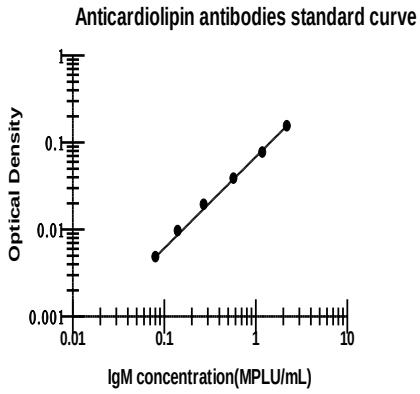

Fig.2 .Comparison between HCV-RNA concentration (IU/ml) of low (<106) and high (> 106) patients

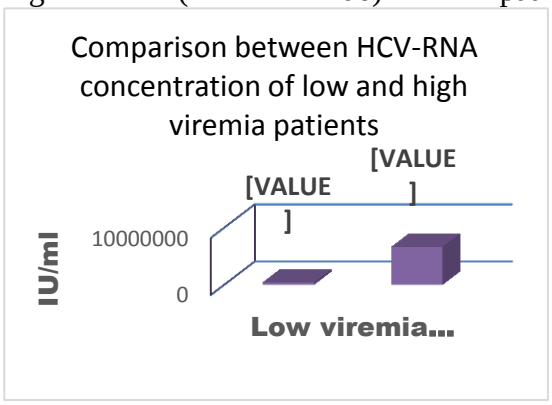

Fig (3): show the aCLPM in the studied 2 groups: it was found that the mean of GGT was higher in Group *II* that have high viremia (HCV –RNA) > 10^6 IU/ml. than in Group *I* that have low viremia (HCV –RNA) < 10^6 IU/ml . With Statistics Significantly different (P =0.0001

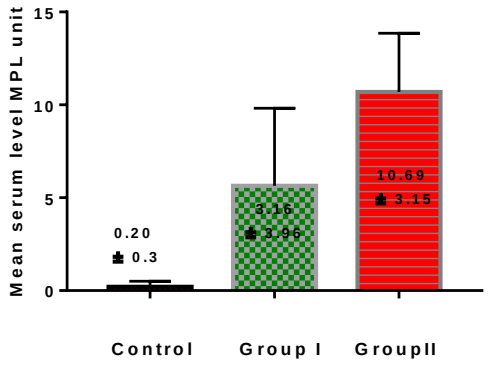

Fig.(4): Comparison between serum levels of ALT(U/L) in low, high viremia patients and controls

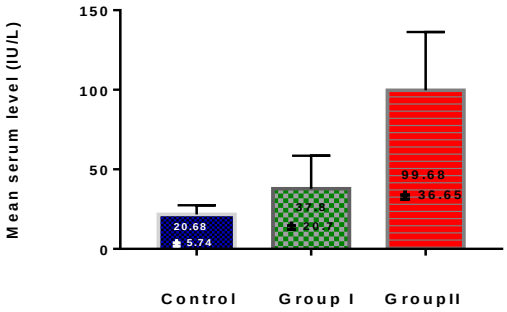

Fig. (5): Comparison between serum level of AST(U/L) in low, high viremia patients and controls

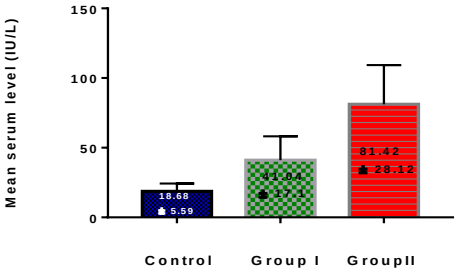

Fig.(6): Comparison between serum level of □-GT(U/L) in low, high viremia patients and controls

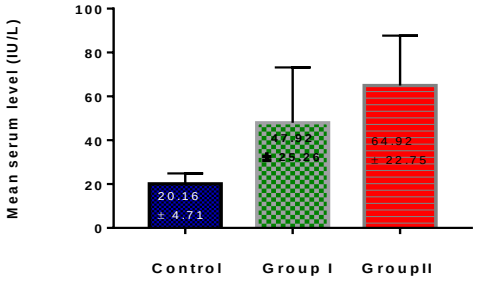

Fig. (7) Correlation between Serum levels of aCLP M (MPL/U) and HCV-PCR value in group II (HCV – PCR value > 1000000)

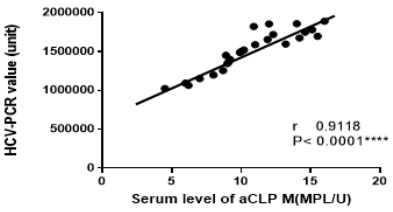

Fig.(8) Correlation between serum levels of aCLP M (MPL/U) and HCV-PCR in group I (HCV-PCR value < 1000000)