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Antiphospholipid Syndrome and HCV Infection

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The research work was conducted to investigate the issue of anti-cardiolipin antibodies (ACAs) and HCV infection, two groups of patients had been selected; the group I (HCV group) comprised 25 patients proved to have HCV infection by PCR test and group II (thrombotic group) comprised 25 patients presented with different thrombotic events. In addition, 25 subjects were selected as a control group. For all patients, estimation of ACA-IgG was done, where 2 samples were taken and the first (initial) sample was at the start of the work. With recent thrombosis, the initial sample was withdrawn before starting heparin. The second (follow-up) sample was taken at least 2 months after the initial one. Results showed that 40% (10 / 25) of group I cases have persistently elevated ACA-IgG and further 40% of them (4 / 25) have the propensity for thrombosis, which was rather predominantly venous in nature. Fifty two percent of unexplained thrombotic cases in group II are ACA-IgG positive; thus the anti-phospholipid syndrome (APS) can account for a good percentage of unexplained thrombosis. Also, there was a significant reduction in platelet count with the rise of ACA-IgG in both groups. Therefore, the laboratory evaluation of APS is suggested to pass in two steps, catching isolated thrombocytopenia, waiting for the assessment of the ACAs as a more specific marker. Finally we can concluded that, the APS is an important additional extra-hepatic feature of HCV and should be anticipated and considered in the differential diagnosis of the different systemic features of this common hepatotropic virus. Advice to screening for the presence of APS in cases with HCV infection by ACA-IgG to be early submitted for antiviral therapy, also we suggested a further work to evaluating its probable clearance after effective successful antiviral therapy.

Key words: antiphospholipid, anti-cardiolipin, hepatitis C, thrombosis, thrombocytopenia

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Introduction

APS is a multisystem disorder which is characterized by persistently elevated anti-phospholipid antibodies, arterial and/or venous thrombosis, thrombocytopenia or recurrent spontaneous abortions (George, 1997). The anti-phospholipid antibodies have been reported in systemic lupus erythematosus and other autoimmune disorders, but are being increasingly detected in patients without underlying autoimmune disease (Asherson *et al.*, 1989). The anti-phospholipid antibodies appeared to be the most common acquired blood protein disorder causing thrombosis (McWorth-Young, 1990).

Cardiolipin is a member of the family of phospholipids and is considered now the most useful antigen used for the phospholipid assay. Furthermore, it showed the strongest association with symptomatic disease (Asherson *et al.*, 1989) and the presence of these antibodies may be an indicator of a prethrombotic state in patients with no evidence of autoimmune disease (McCrea *et al.*, 1991).

The ACAs have been detected in a variety of infectious diseases, the particularly of viral origin, such as human immun. deficiency virus infection, mumps and hepatitis A (McNeil *et al.*, 1991) but there are no data concerning the prevalence of ACAs in HCV infection. These considerations have triggered us to investiage the issue of ACAs and HCV infection.

Materials and Methods

The research was performed on 50 patients, selected from the Inpatient Departments of Internal Medicine, Vascular surgery and Neurology, Mansoura University Hospital. They were divided into two groups (I & II) as compared to a control group.

Group I (HCV group): It included 25 patients, 14 males (56%) and 11 females (44%) proved by the ELISA test (3rd generation) to be positive for HCV antibodies. Their age ranged from 27-42 years (mean ± SD; 36.44 ± 4.29 years).

Group II (thrombotic group): It included 25 patients presented with thrombotic events (9 presented with DVT, 8 with cerebral infection, 1 with superior vena caval thrombosis and 7 patients with mixed thrombotic presentations). They were 15 (60%) males and 10 females (40%). Their age ranged from 18 to 41 years (mean ± SD; 32.48 ± 7.17 years).

Exclusion criteria

1 Patients with viral hepatitis other than HCV.
2 Patients with known collagen vascular or autoimmune diseases such as SLE and rheumatoid arthritis.
3 Pregnant ladies or those in the postpartum period or those with a history of taking a hormonal contraception.
4 Patients with ischemic heart disease, hypertension, valvular heart disease, heart failure, DM, renal failure, or malignant disease.
5 Patients with a history of recent trauma or surgery.
6 Old people, heavy smokers and morbidly obese patients.

Control group: It included 25 healthy volunteers, 14 males (56%) and 11 females (44%). Their age ranged from 19 to 40 years (mean ± SD 33.04 ± 7 years).

All the participants were subjected to thorough history taking with special stress on history of thrombosis (recent or remote) and history of symptoms suggesting hepatic insult. Thorough physical examination was done to evaluate the thrombotic event as well as the general and local signs suggestive of hepatic infection. Laboratory investigations were done including the liver function tests, lipid profile, serum creatinine, complete blood picture, prothrombin time, activated partial thromboplastin time.

Immunological studies including ANA, Anti ds-DNA, RF, ELISA for HCV antibodies, and lastly ACA-IgG, where 2 samples were taken. The first initial sample was taken before starting heparin in cases with recent thrombosis and the second follow up sample was taken at least 2 months after the initial one. According to Harris *et al.* (1994), values of ACA-IgG that exceed 10 GPL are considered positive results.

Radiologic studies were done according to the type of thrombotic event.

Statistical analysis: Values were expressed as counts & percentages (compared by the Chi-Square or Fisher's Exact test), or mean ± SD (compared by Independent-Samples T-test). Multiple means were compared by the One Way Analysis of Variance (ANOVA). All statistical calculations were performed using SPSS 7.5 for windows (SPSS Inc., 1989 - 1996).

Results

Age and sex according to ACA positivity: Twenty-three cases were found to be positive for ACA-IgG versus 52 ACA negative cases. They were 14 males and 9 females versus 29 males and 23 females in ACA negative cases (Chi-Square = 0.17, p = 0.68; Fisher's exact test: p = 0.8; as compared to ACA negative cases). Their mean age ± SD was 35.13 years ± 6.16 versus 33.48 ± 6.58 for ACA negative cases (t = 1.021, p = 0.311). Table 1 showed a statistically higher prevalence of ACA-IgG positivity in cases with HCV (group I) as well as those with thrombotic manifestations (group II) versus control.

Table 1: ACA- IgG in the studied groups

(n = 25)	ACA positive (%)	ACA negative (%)
Group I	10 (40)	15 (60)
Group II	12 (48)	13 (52)
Control	1 (4)	24 (96)

Chi-Square = 12.918, p = 0.002
values in parenthesis showed percentage

Table 2: Clinical characteristics of group I cases

Characteristic	Count (%) within total group (n = 25)	Count (%) within ACA positive cases (n = 10)
Fetor hepaticus	7 (28)	3 (30)
Flapping tremor	7 (28)	2 (20)
Jaundice	5 (20)	1 (10)
Bleeding tendency	7 (28)	4 (40)
Finger clubbing	4 (16)	1 (10)
Collaterals	7 (28)	4 (40)
Ankle edema	8 (32)	2 (20)
Leuconychia	3 (12)	1 (10)
Pallor	5 (20)	2 (20)
Spider nevi	3 (12)	1 (10)
Palmar erythema	6 (24)	1 (10)
Enlarged parotids	5 (20)	1 (10)
Pulmonary hypertension	4 (16)	1 (10)
Thrombotic Manifestation	4 (16)	4 (40)
Liver size:		
- Shrunken:	21 (84)	8 (80)
- Average:	3 (12)	1 (10)
- Enlarged:	1 (4)	1 (10)
Splenomegaly:		
- Mild:	18 (72)	9 (90)
Moderate:	7 (28)	1 (10)

values in parenthesis showed percentage

Clinical characteristics of group I: Table 2 showed the clinical manifestations in group I cases. Fisher's exact test revealed that thrombotic presentation is the only one of the listed manifestations significantly more frequent with ACA positivity (p = 0.017).

Clinical presentations of group II: Table 3 showed the clinical characteristics of group II cases. None of the listed presentations had a statistically significant more frequency within ACA positive cases.

Table 3: Clinical presentations of group II cases

Presentation	Count (%) within total group (n = 25)	Count (%) within ACA positive cases (n = 12)
Deep vein thrombosis	10 (40) Recurrent: 5 (20)	5(41.7) - Recurrent: 4 (33.3)
Pulmonary thromboembolism	4 (16)	3 (25)
Cerebral infarction	9 (36)	2 (16.7)
Superior vena caval thrombosis	1 (4)	1 (8.3)
Recurrent spontaneous abortions	2 (8)	2 (16.7)

values in parenthesis showed percentage

Correlation between ACA-IgG levels and laboratory data in group I cases: Both initial and follow up ACA-IgG in HCV cases were significantly negatively correlated with initial as well as follow up platelet counts and significantly positively correlated with follow up APTT (Table 4).

Table 4: Pearson's correlation between ACA-IgG levels (GPL) and different laboratory findings in group I cases

Parameter	Initial ACA IgG		Follow up ACA-IgG	
	r	p	r	p
Serum albumin	- 0.027	0.899	0.119	0.572
Serum bilirubin	- 0.221	0.289	- 0.403	0.046
Initial AST	- 0.042	0.84	- 0.254	0.221
Follow up AST	- 0.026	0.903	- 0.27	0.192
Initial ALT	0.045	0.831	- 0.278	0.179
Follow up ALT	- 0.06	0.776	- 0.301	0.144
Hemoglobin	0.163	0.436	0.194	0.352
WBCs	0.591	0.002	0.266	0.199
Initial platelet	- 0.736	< 0.001	- 0.768	< 0.001
Follow up platelet	- 0.634	0.001	- 0.741	< 0.001
Initial PT	- 0.159	0.448	- 0.394	0.051
Follow up PT	- 0.108	0.607	- 0.174	0.406
Initial APTT	0.182	0.383	0.185	0.376
Follow up APTT	0.696	< 0.001	0.726	< 0.001

Table 5: Pearson's correlation between ACA-IgG levels (GPL) and different laboratory findings in group II cases

Parameter	Initial ACA- IgG		Follow up ACA- IgG	
	r	p	r	p
Hemoglobin	- 0.017	0.937	0.025	0.906
WBC	0.11	0.6	0.039	0.854
Initial platelet	- 0.537	0.006	- 0.483	0.014
Follow up platelet	- 0.617	0.001	- 0.538	0.005
Initial PT	- 0.091	0.665	0.041	0.517
Follow up PT	0.136	0.845	0.144	0.493
Initial APTT	0.099	0.639	0.05	0.812
Follow up APTT	0.375	0.064	0.434	0.03

Correlation between ACA-IgG levels and laboratory data in group II cases: Table 5, both initial and follow up cases were significantly negatively correlated with initial as well as follow up platelet counts in cases presenting with thrombotic manifestations. In addition, non-parametric (Spearman's) correlation revealed a significantly positive correlation between Anti-HCV antibodies and both initial ACA-IgG ($r_s = 0.642$, $p = 0.001$) and its follow up levels ($r_s = 0.63$, $p = 0.001$).

Stepwise linear regression analysis for ACA-IgG: Table 6 showed that in cases of presenting with HCV, the most important predictor variables for ACA-IgG are presence of thrombotic manifestations as well as initial low platelet count. On the other hand, the most important predictors of ACA-IgG in cases presenting with thrombosis are positive test for anti-HCV antibodies as well as a high platelet count on follow up.

Table 6: Stepwise regression analysis for ACA IgG:

Predictor	Initial ACA IgG		Follow up ACA IgG	
	β	p	β	p
Group I (cases presenting with HCV)				
Thrombotic manifestations	0.736	<0.001	0.777	<0.001
Initial platelet	0.736	<0.001	0.768	<0.001
Group II (cases presenting with thrombosis)				
Anti-HCV	0.694	<0.001	0.647	<0.001
Follow up platelet	0.617	0.001	0.538	0.005

Discussion

HCV infects at least 200 million people world-wide, and it is more common in the Mediterranean countries and in Africa including Egypt (Alter, 1997). It has a wide spectrum of clinical manifestations including hepatic, hematologic, renal, dermatologic as well as autoimmune features which further certify that the wide variety of potential clinical presentations of HCV. More informations are needed to determine that whether many autoimmune diseases as the APS is a part of growing clinical spectrum or just coincidental with chronic liver disease(Dickson, 1997)

Prieto *et al.* (1996) concluded that the prevalence of ACAs is increased in HCV infection and that HCV should be regarded as a possible causative factor in the APS. In this research, 40% (10/25) of HCV cases have persistently elevated ACA-IgG and 16% of them (4/25) have thrombotic complications which are rather predominantly venous in nature.

In the thrombotic group, 12 patients (48%) have persistent elevations of ACA-IgG. APS can account for a good percentage of unexplained thrombotic epidodes with no age or sex predominance. Thus, our findings showed that, nearly half of the young, unexplained thrombotic cases are ACA-IgG positive and nearly half of them are HCV positive. Furthermore, the thrombotic HCV cases presented mainly with DVT or thrombosis at multiple sites (mixed), an observation that may abort the tendency of the HCV-positive thrombotic syndrome to create arterial thrombosis like coronary or cerebral and considered HCV infection , in this situation , as a latent state. The remaining thrombotic cases with persistent elevation of ACA-IgG and negative HCV serology are in the need for further investigation and research for the associated creative cause.

Regardings the thrombotic group , our plan for selection of the cases that have excluded other etiologies of APS other than HCV, did not allow us to study thoroughly the clinical features of APS of different etiologies, so we can no more mentioned the different clinical profiles of this syndrome.

The result revealed that there was a significant reduction in the platelet count with the rise of ACA-IgG in the HCV-positive group and the thrombotic group in initial as well as in the follow up samples. Also, the HCV-positive groups has revealed that there are 10 patient (40%) with persistent elevation of ACA-IgG level, 9 patients (36%) have marked, selective

thrombocytopenia and one patient (4%) have moderate thrombocytopenia associated with anaemia. In comparison, the thrombotic group has 12 patients (48%) with persistent elevation of ACA-IgG; seven with marked selective thrombocytopenia, two with moderate thrombocytopenia associated with anemia, and three with moderate thrombocytopenia.

The probability of the presence of pancytopenia as a feature of the hypersplenism in group I of chronic advanced HCV infection obliged us to look for the isolated thrombocytopenia as an additional marker of APS. On the other hand, although thrombocytopenia can acquire an additional cause with HCV infection, yet, we can suggested a trial for estimation of the isolated thrombocytopenia as an easy first usher of APS, in these cases, waiting for the ACAs, the other laboratory composite, as a next and a more specific marker.

APAs react against phospholipids used in clotting tests as APTT resulting in prolonged coagulation times (Green, 1987). These antibodies, probably, inhibit the assembly of clotting factors at the phospholipid surface at the prothrombin activator complex stage of the clotting cascade, thus, prolonging the *in vitro* clotting (Harris *et al.*, 1985).

In this research work, the APTT showed a significant prolongation with the increase of ACA-IgG in initial samples, in both groups (the HCV-positive and the thrombotic) as well as in total persistently elevated ACA-IgG group and this significantly increase persists with follow up of the same patient groups. This correlative study have revealed that these data of evaluation are truly valid and thus the components of syndrome have maintained their correlation as well as APTT and ACAs and further follow up of the unexplained thrombotic cases entails that this syndrome is not probably a transient hematological state but it has started to continue at least four months.

The correlation between ACAs and the biochemical marker of HCV, ALT has been fairly maintained by a little positive correlation in the frank HCV positive cases, but it has been lost in the second latent cases, a finding which goes with the statement which entailed that there are many cases of the asymptomatic HCV infection with multisystem implications inspite of persistently normal ALT and this raise the discussion about the radical treatment of latent cases to limit their late bad sequalae.

Considering the big magnitude of this syndrome associated with HCV infection, we advice a screening for its presence in the HCV –positive patients by ACA-IgG to be early submitted to antiviral therapy. Also, we suggested a further investigation evaluating its probable clearance after effective antiviral therapy and the laboratory evaluation of APS to pass in two steps; first catching isolated thrombocytopenia, waiting for the ACAs as a more specific marker. Furthermore, thrombocytopenia which is famously considered as an usher for haemorrhage? could it be an usher for thrombosis; it waits for ACA assessment.

In conclusion, isolated thrombocytopenia adjuncted to HCV infection, in the absence of hypercellular bone marrow, and waiting for the more specific rise of ACA, can be an usher for APS.

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