Study of Beta 2 Glycoprotein 1 Antibodies in HCV Positive Patients on HD and Its Relation to Vascular Access Thrombosis

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Abstract: Background: Although the precise physiological role of B2-GPI is not known. B2-GPI has been shown to inhibit intrinsic pathway activation and prothrombinase activities. Hemodialysis access failure is a leading cause of morbidity and hospitalization for patients with end-stage renal disease. **Patients and methods:** our study was conducted on forty patients under regular hemodialysis randomly selected from hemodialysis units of Ain Shams University Hospitals. Patients were divided into Group A: 20 patients with positive hepatitis C virus antibodies, and group B: 20 patients with negative hepatitis C virus antibodies. All patients were subjected to: full clinical examination, routine CBC, ESR and quantitative CRP, routine chemistry including (BUN, serum creatinine), serum Na, serum K, serum Ca, serum PO4, serum albumin, and total proteins. Liver enzymes (AST, ALT), routine coagulation profile (PT, INR, PTT), HBsAG and HCVab, B2 glycoprotein I antibodies IgM, IgG titers by ELISA and assessment of fistula flow by Doppler ultrasound were also done. **Results:** We found that the frequency of B2IgM and B2IgG positive or borderline in group A was 20 % (4 patients), 25% (5 patients) respectively while the frequency of B2 IgM and B2IgG in group B was 30% (6/20 patients), 35% (7/20 patients) respectively. No relation was found between B2-glycoprotein I antibodies and HCV in prevalent hemodialysis patients. Our study revealed the frequency of B2 IgM was 40% and B2 IgG was 40% positive or borderline in patients with AVF with positive history of vascular access occlusion. Elevated B2GPI IgM titre is associated with decreased fistula volume of flow by Doppler.
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Key words: Beta2glycoprotein1 antibodies – HCV- HD – Vascular access thrombosis.

1. Introduction:

Beta 2 glycoprotein 1 (beta 2 GP1, also called apolipoprotein H) is a 326 amino acid synthesized by hepatocytes, endothelial cells and trophoblast cells (1).

B_2GP_1, *in vitro* studies suggest that it likely functions as a natural anticoagulant. B_2GP_1 has been shown to inhibit intrinsic pathway activation and prothrombinase activities on the surface of activated platelets and synthetic phospholipids vesicles. B_2GP_1 also inhibits the activity of activated protein C on procoagulant surfaces. B_2GP_1 inhibits ADP-induced platelet aggregation (2).

B_2GP_1 has become well-known as a co-factor for anticardiolipin auto-antibodies. Autoantibodies against B_2GP_1 are described for various autoimmune disease. The presence of anti-B_2GP_1 antibodies can be related to the development of arterial and venous thromboses, venous thromboembolism, thrombocytopenia and fetal loss. Anti-B_2GP_1 antibodies are found in the immunoglobulin classes IgG, IgM and IgA (3).

Anti-B_2GP_1 IgG antibody titers correlate well with the clinical status of the patients in thrombosis, thromboembolism and repeated fetal loss, while anti-B_2GP_1 IgM antibodies show significant association with thrombosis and thrombocytopenia(3).

Sands *et al.* (2001)(4) showed that haemodialysis patients had elevated anti-B_2GP_1 antibodies and patients with PTFE grafts had elevated antibodies most frequently versus fistulas and tunneled catheters and this study concluded that haemodialysis patients with PTFE grafts frequently have elevated antibodies to beta 2 GP_1 and the presence of elevated antibody levels is associated with an increased thrombotic risk.

Previously, there were attempts to detect anticardiolipin antibodies IgM, IgG in HCV positive hemodialysis patients. However, the relation between its presence and thrombotic events including fistula thrombosis was not proved. Elevated IgM aCL titer was present in 17.4% of chronic HD patients. Results suggest recurrent vascular access thrombosis of synthetic or native fistula may not be caused by elevated IgM-aCL (IgM-anticardiolipin antibody) titer in these patients (5).

Ozmen *et al.* (2009)(6), showed that prevalence of IgG-aCL (IgG-anticardiolipin antibody) in chronic HD patients was 14.6% and no patient had a positive value of the IgM-aCL test.

2. Patients and Methods

This study was conducted on forty patients with ESRD on regular hemodialysis (3 sessions per week) randomly selected from hemodialysis units of Ain Shams University Hospitals between September 2010 and September 2011. The patients were divided into 2 groups:

Group A: 20 patients with positive hepatitis C virus antibodies.

Group B: 20 patients with negative hepatitis C virus antibodies.

We excluded from the study ESRD patients on regular hemodialysis having diabetes mellitus, collagen disorders, coagulation abnormality other than that associated with chronic liver disease including drug induced liver disease. Smokers were excluded from the study.

All patients were subjected to full clinical examination including vascular access examination (synthetic or native fistula or catheter).

Routine complete blood count (CBC), ESR and quantitative CRP, BUN, serum creatinine, serum Na, serum K, serum Ca, serum PO_4, serum albumin, total proteins, liver enzymes (serum ALT, serum AST), routine coagulation profile (PT, INR, PTT), HBsAg and HCVab, B_2 glycoprotein I antibodies IgM, IgG titer by ELISA.

Methods:

All routine investigations were done by conventional methods at Ain Shams University Hospital laboratories.

Beta-2 glycoprotein I estimation principle of the test

Highly purified beta-2 glycoprotein I is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgA immunologically detects the bound patient's antibodies forming a conjugate/antibody/antigen complex washing of the microwells remove unbound conjugate.

An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometricaly at 450 nm.

The amount of colour is directly proportional to the concentration of IgA antibodies present in the original sample.

Interpretation of results

Quality control:

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrators A and F complies with the respective range indicated on the quality control certificate enclosed to each test kit. If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Interpretation of results

In a normal range study with serum samples from healthy blood donors, the following ranges have been established with the anti-beta-2- glycoprotein I test.

Anti-beta-2 glycoprotein I Ab IgM, IgG (U/ml)

Normal: < 5

Borderline: 5-8

Positive: > 8

Statistical analysis of data

T-test and paired t-test were used to compare means of parametric data group while Mann Whitney test was used for the non parametric data.

Pearson chi-square correlation test were used to correlate parametric values and spearman correlations was used for the non parametric data.

3. Results

Participants in group A were 16 females (80%) and 4 males (20%). The available access was natural arteriovenous fistula (AVF) in 17 patients, polytetrafluoroethylene (PTFE) grafts in 2 patients and cuffed catheter in 1 patient.

Participants in group B were 13 females (65%) and 7 males (35%), two patients had a history of cerebrovascular stroke and B_2-GPI was negative, one patient with DVT with borderline B_2 IgG-1. Two patients died after termination of our study (one was due to pulmonary edema and the other was due to hematemesis).

HCV PCR done for patients with negative HCV antibody with positive B_2-GPI antibody, was found to be negative. Patients were further classified into 2 equal groups according to vascular access occlusion:

First group: Patients having positive history of vascular access occlusions (group C).

Second group: Patients having negative history of access (occlusion (group D).

4. Discussion

Antiphospholipid (aPL) antibodies (Abs) were discovered in 1952 in patients suffering from systemic lupus erythematosus (SLE), it has the ability to prolong coagulation time in vivo from which the name lupus anticoagulant (LA) arose (7).

Later on, it was discovered that aPL do not act as anticoagulants in vivo, and can be found not only in SLE patients but also in apparently healthy individuals (8).

At the beginning of the 1990s, beta-2 glycoprotein 1 (B2-GPI) was shown to be a major antigen for anti-phospholipid antibodies. Although anticardiolipin antibodies (aCL) Abs and Abs against B_2-GPI (anti-B_2-GPI Abs) often coexist, they are not identical.

Interestingly, the highest risk of thrombosis was associated with the presence of both LA with anti-B_2GPI.

Table (1): Comparison of quantitative variables in group (A) and group (B)

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Age in years	HCV+ve (group A)	20	47.65	±12.571	-0.147	>0.05	NS
	HCV-ve (group B)	20	47.05	±13.153			
Systolic B.P. in mmHg	HCV+ve (group A)	20	124.500	±19.86136	0.793	>0.05	NS
	HCV-ve (group B)	20	130.000	±23.84158			
Diastolic b.P. in mmHg	HCV+ve (group A)	20	77.00	±10.31095	0.000	>0.05	NS
	HCV-ve (group B)	20	77.000	±11.74286			
WBcs 10^6/cmm	HCV+ve (group A)	20	6.220	±2.3761	0.376	>0.05	NS
	HCV-ve (group B)	20	6.500	±2.3317			
HGB in mmHg	HCV+ve (group A)	20	10.450	±1.9503	-0.986	>0.05	NS
	HCV-ve (group B)	20	9.895	±1.5906			
PLT 10^6/cmm	HCV+ve (group A)	20	218.45	±68.241	0.899	>0.05	NS
	HCV-ve (group B)	20	239.10	±76.730			
BUN mg/dl	HCV+ve (group A)	20	61.35	±27.601	0.841	>0.05	NS
	HCV-ve (group B)	20	67.85	±20.790			
Creat mg/dl	HCV+ve (group A)	20	7.74	±2.223	2.233	0.032	**S**
	HCV-ve (group B)	20	9.45	±2.606			
Alb gm/dl	HCV+ve (group A)	20	3.40	±0.592	1.854	0.071	NS
	HCV-ve (group B)	20	3.68	±0.299			

*** t-test was used**

Table (2): Comparison of quantitative variables in group (A) and group (B) (Cont..)

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Na meq/l	HCV+ve (group A)	20	136.75	5.025	1.007	>0.05	NS
	HCV-ve (group B)	20	138.10	3.275			
K meq/l	HCV+ve (group A)	20	5.205	0.7287	0.414	>0.05	NS
	HCV-ve (group B)	20	5.315	0.9382			
Ca mg/dl	HCV+ve (group A)	20	8.575	0.8058	0.728	>0.05	NS
	HCV-ve (group B)	20	8.790	1.0467			
Po4 mg/dl	HCV+ve (group A)	20	4.295	1.5463	2.505	0.017	**S**
	HCV-ve (group B)	20	5.415	1.2671			
Ast	HCV+ve (group A)	20	19.40	6.44	0.259	> 0.05	NS
IU	HCV-ve (group B)	20	21.05	27.757			
Alt	HCV+ve (group A)	20	17.30	5.222	-.221	>0.05	NS
IU	HCV-ve (group B)	20	16.35	18.488			
Total bilirubin	HCV+ve (group A)	20	0.69	0.268	1.493	>0.05	NS
mg/dl	HCV-ve (group B)	20	0.83	0.323			
INR	HCV+ve (group A)	20	1.0035	0.05284	-1.144	>0.05	NS
	HCV-ve (group B)	20	0.9850	0.04936			
PTT sec	HCV+ve (group A)	20	35.805	7.6490	0.137	>0.05	NS
	HCV-ve (group B)	20	36.200	10.3417			
Doppler flow	HCV+ve (group A)	20	740.865	134.3721	-.624	>0.05	NS
ml/min n=17	HCV-ve (group B)	20	706.824	180.2599			
Intradialytic	HCV+ve (group A)	20	2.7250	1.19731	-1.167	>0.05	NS
weight gain	HCV-ve (group B)	20	2.300	1.10501			

*** t-test was used**

Table (3): Comparison of group (A) and group (B) as regard ESR and CRP

	HCV state	N	Median	Std. Error of Mean	Mann Whitney*	*P*-value	Sig.
Duration of	HCV+ve (group A)	2	14	10	0.827	>0.05	NS
hemodialysis n =20	HCV-ve (group B)	3	13	7	0.694		
ESR 1st hour	HCV+ve (group A)	20	47.75	46.693	199.000	>0.05	NS
	HCV-ve (group B)	20	38.60	31.149			
CRP mg/dl	HCV+ve (group A)	20	11.70	20.275	165.000	>0.05	NS
	HCV-ve (group B)	20	9.30	5.667			

***Mann Whitney u test was used was used**

Table (4): Comparison of group (A) and group (B) as regard B2 IgM and B2 IgG:

		HCV state				Value of chi-square test*	*P* value > 0.05 NS < 0.05 S	Sig.
		Positive A		Negative B				
		No.	Percent	No.	Percent			
B2 IgM u/ml	Negative	16	80%	14	70%	0.533	0.465	NS
	Positive	4	20	6	30			
B2-IgG u/ml	Negative	15	75	13	65	0.476	0.490	NS
	Positive	5	25	7	35			

*** Chi square test was used**

No statistical significance as regard correlation between b2 glycoprotein antibodies IgM, IgG and HCV state (positive or negative) (*p* value > 0.05).

Table (5): Comparison of group (A) and group (B) as regard B2 IgM and B2 IgG tire.

	HCV State				Value of chi-square test*	*P* value > 0.05 NS < 0.05 S	Sig.
	Positive A		Negative B				
	Median	Std. error	Median	Std. error			
B2 IgM titre u/ml	3.000	0.6729	4.000	1.1056	143.000	.120	NS
B2-IgG titre u/ml	3.75	0.268	4.00	0.447	161.500	.293	NS

*** Mann Whitney u test was used**

No statistical significance as regard correlation between b2 glycoprotein antibodies IgM, IgG and HCV state (positive or negative) (*p* value > 0.05).

Table (6): Risk estimation via odds ratio for: B2 IgM & B2 IgG positive or borderline

	Odds ratio	95% confidence interval	
		Lower	Upper
B2 IgM u/ml (negative/positive)	0.583	0.136	2.498
B2 IgG u/ml (negative/positive)	0.619	0.158	2.429

This table 3.10 shows that:

B2 IgM negative is more likely to be among group A "HCV +ve patients" than B "HCV –ve patients" by a ratio of 0.583. B2 IgG negative is more likely to be among group group A "HCV +ve patients " than B "HCV –ve patients" by a ratio of 0.619.

Table (7): Correlation between B2-IgM, B2-IgG and other variables in HCV +ve (group A)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	-0.027	0.911	NS	0.103	0.667	NS
Duration of hemodialysis "n=30" in years	-0.081	0.735	NS	0.195	0.41	NS
Systolic BP "n=20" in mmHg	-0.329	0.157	NS	0.197	0.406	NS
Diastolic BP "n = 20" in mmHg	-0.241	0.306	NS	0.276	0.239	NS
Wbc "n=20" 10^6/cmm	-0.342	0.14	NS	-0.112	0.639	NS
Hb "n=20" gm/dl	0.229	0.332	NS	0.251	0.286	NS
Plt "n=20" 10^6/cmm	-0.067	0.779	NS	-0.062	0.794	NS
BUN "n=20" mg/dl	0.168	0.48	NS	-0.507(*)	0.027	**S**
Cr 'n=20" mg/dl	-0.167	0.481	NS	-0.494(*)	0.027	**S**
Albumin "n=20" gm/dl	-0.186	0.432	NS	0.22	0.352	NS
Na "n=20" meq/L	0.142	0.551	NS	-0.219	0.354	NS
K 'n = 20" meq/L	0.142	0.551	NS	-0.219	0.354	NS
Ca "n=20" mg/dl	0.14	0.557	NS	0.383	0.096	NS
Po4 "n=20" mg/dl	0.248	0.291	NS	-0.046	0.849	NS
Ast IU	0.391	0.089	NS	-0.108	0.651	NS
Alt IU	0.281	0.23	NS	0.039	0.869	NS

* Spearman test was used

Table (8): Correlation between B2-IgM, B2-IgG and other variables in HCV +ve (group A) (Cont…)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Bil total mg/dl	0.463	0.04	S	-0.018	0.941	NS
INR	0.348	0.133	NS	0.012	0.959	NS
PTT sec	0.121	0.61	NS	0.176	0.459	NS
ESR 1st hour	0.225	0.341	NS	0.057	0.81	NS
CRP mg/dl	-0.258	0.272	NS	0.07	0.77	NS
Doppler flow ml/min n=17	-0.209	0.420	NS	-0.271	0.292	NS
B2-IgG u/ml	0.142	0.55	NS			

* Spearman test was used

Table (9): Correlation between B2-IgM, B2-IgG and other variables in HCV -ve (group B)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	-0.187	0.431	NS	-0.31	0.183	NS
Duration of hemodialysis "n=30" in years	0.396	0.084	NS	-0.198	0.402	NS
Systolic BP "n=20" in mmHg	0.142	0.55	NS	0.437	0.054	NS
Diastolic BP "n = 20" in mmHg	-0.045	0.849	NS	0.312	0.181	NS
Wbc "n=20" 10^6/cmm	0.21	0.35	NS	0.373	0.106	NS
Hb "n=20" gm/dl	-0.119	0.617	NS	0.092	0.699	NS
Plt "n=20" 10^6/cmm	0.252	0.283	NS	0.122	0.61	NS
BUN "n=20" mg/dl	0.407	0.075	NS	0.246	0.296	NS
Cr 'n=20" mg/dl	0.127	0.594	NS	-0.221	0.348	NS
Albumin "n=20" gm/dl	-0.301	0.197	NS	-0.127	0.593	NS
Na "n=20" meq/L	-0.136	0.567	NS	-0.016	0.945	NS
K 'n = 20" meq/L	0.151	0.526	NS	-0.243	0.302	NS
Ca "n=20" mg/dl	0.278	0.235	NS	0.169	0.477	NS
Po4 "n=20" mg/dl	-0.108	0.651	NS	0.017	0.942	NS
Ast IU	0.198	0.403	NS	0.224	0.342	NS
Alt IU	0.325	0.162	NS	0.169	0.476	NS
Bil total mg/dl	0.161	0.497	NS	0.221	0.35	NS
INR	-0.174	0.464	NS	-0.417	0.068	NS

* Spearman test was used

Table (10): Correlation between B2-IgM, B2-IgG and other variables in HCV -ve (group B) (Cont…)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
PTT sec	0.046	0.848	Ns	-0.148	0.533	NS
ESR 1st hour	0.318	0.172	NS	-0.181	0.444	NS
CRP mg/dl	0.062	0.796	NS	0.03	0.901	NS
Doppler flow ml/min n=17	-0.254	0.326	NS	-0.028	0.914	NS
B2-IgG u/ml	0.26	0.268	NS	0.26	0.268	NS

* Spearman test was used

Table (11): Comparison of quantitative variables in patients with positive and negative history of vascular access occlusion

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Age in years	Positive	20	45.45	12.672	0.945	>0.05	NS
	Negative	20	49.25	12.769			
Systolic B.P. in mmHg	Positive	20	124.000	21.373	0.940	>0.05	NS
	Negative	20	130.500	22.354			
Diastolic b.P. in mmHg	Positive	20	76.00	10.9544	0.575	>0.05	NS
	Negative	20	78.00	11.050			
WBcs 10^6/cmm	Positive	20	6.550	2.5264	-0.511	>0.05	NS
	Negative	20	6.170	2.1599			
HGB in mmHg	Positive	20	9.985	1.4339	0.662	>0.05	NS
	Negative	20	10.360	2.0894			
PLT 10^6/cmm	Positive	20	217.70	71.506	-0.966	>0.05	NS
	Negative	20	239.85	73.467			
BUN mg/dl	Positive	20	64.70	24.883	-0.026	>0.05	NS
	Negative	20	64.50	24.436			
Creat mg/dl	Positive	20	8.47	2.747	0.295	>0.05	NS
	Negative	20	8.71	2.386			
Alb gm/dl	Positive	20	3.50	0.358	0.486	>0.05	NS
	Negative	20	3.58	0.590			
Intradialytic weight gain	Positive	20	2.600	1.11921	-0.473	>0.05	NS
	Negative	20	2.425	1.21693			

*** t-test was used**

Table (12): Comparison of quantitative variables in patients with positive and negative history of vascular access occlusion (cont…)

	HCV state	N	Mean	SD	t*	*P*-value	Sig.
Na meq/L	Positive	20	136.90	4.941	0.779	>0.05	NS
	Negative	20	137.95	3.456			
K meq/L	Positive	20	5.325	0.8608	-0.490	>0.05	NS
	Negative	20	5.195	0.8172			
Ca mg/dl	Positive	20	8.755	1.1532	-0.489	>0.05	NS
	Negative	20	8.610	0.6545			
Po4 mg/dl	Positive	20	4.655	1.2458	0.837	>0.05	NS
	Negative	20	5.055	1.7380			
Ast IU	Positive	20	23.55	27.657	-1.056	>0.05	NS
	Negative	20	16.90	5.281			
Alt IU	Positive	20	20.05	18.193	-1.547	>0.05	NS
	Negative	20	13.60	4.083			
Total bilirubin mg/dl	Positive	20	0.82	0.341	-1.270	>0.05	NS
	Negative	20	0.70	0.250			
INR	Positive	20	0.9890	0.05848	0.642	>0.05	NS
	Negative	20	0.9995	0.04395			
PTT sec	Positive	20	38.665	11.6042	-1.940	>0.05	NS
	Negative	20	33.340	3.9948			
Doppler flow ml/min n=17	Positive	20	383.514	177.9665	1.260	>0.05	NS
	Negative	20	752.075	139.2072			

*** t-test was used**

Table (13): Comparison of patients with positive and negative history of vascular access occlusion as regard ESR and CRP:

	History of vascular access occlusion	N	Median	Std. error of mean	t*	*P* value	Sig.
ESR 1st hour	Positive	20	46.85	41.808	-0.584	>0.05	NS
	Negative	20	39.50	37.658			
CRP mg/dl	Positive	20	9.60	6.573	0.382	>0.05	NS
	Negative	20	11.40	20.033			

*t-test was used

Table (14): Comparison of patients with positive and negative history of vascular access occlusion as regards B2-IgM and B2-IgG

		Vascular access state				*Value of chi-square test*	*P* value	Sig.
		Positive history vascular access occlusion "n=20"		Negative history of vascular access occlusion "n=20"				
		No.	Percent	No.	Percent			
B2 IgM u/ml	Negative < 5	12	60	18	90	4.800	0.028	**S**
	Positive ≥5	8	40	2	10			
B2-IgG u/ml	Negative < 5	12	60	16	80	1.905	0.168	NS
	Positive ≥5	8	40	4	20			

* Chi-square test was used

Table (15): Comparison of group (A) and group (B) as regard B2 IgM and B2 IgG tire.

	Vascular access state				*Value of Mann-Whitney U	*P* value	Sig.
	Positive history vascular access occlusion "n=20"		Negative history vascular access occlusion "n=20"				
	Median	Std. error	Median	Std. error			
B2 IgM u/ml	4.000	1.1349	3.000	0.5781	121.000	0.031	**S**
B2-IgG u/ml	4.00	0.335	4.00	0.414	176.00	0.512	NS

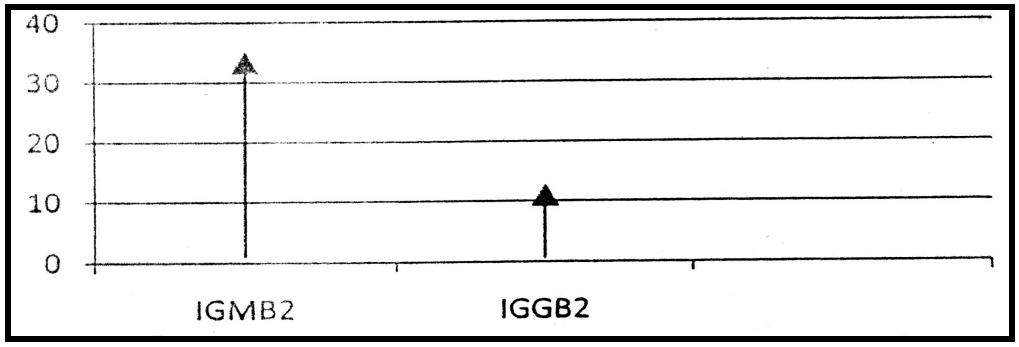
* Mann Whitney u test was used

Table (16): Risk estimation via odds ratio for B2 IgM & B2 IgG

	Odds ratio	95% confidence interval	
		Lower	Upper
B2 IgM u/ml (negative/positive)	6.00	1.082	33.274
B2 IgG u/ml (negative/positive)	2.667	0.648	10.972

B2 IgM positive is more likely to be among patients with positive history of vascular access occlusion than patients with negative history of vascular access occlusion by a ratio of 6.

So patients with positive B2 IgM have the risk of thrombosis 6 times than individuals with negative B2-IgM. B2 IgG positive more likely to be among patients with positive history of vascular access occlusion than patients with negative history of vascular access occlusion "by a ratio of 2.3.



Odds ratio for: B2 IgM and B2 IgG in patients with positive and negative history of vascular access occlusion.

Table (17): Correlation between B2-IgM and other variables in patients with positive history of vascular access occlusion (group C)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	0.016	0.945	NS	0.137	0.565	NS
Duration of hemodialysis "n=30" in years	0.112	0.637	NS	-0.357	0.122	NS
Systolic BP "n=20" in mmHg	-0.062	0.795	NS	0.336	0.148	NS
Diastolic BP "n = 20" in mmHg	-0.05	0.834	NS	0.423	0.063	NS
Wbc "n=20" 10^6/cmm	-0.004	0.986	NS	0.176	0.459	NS
Hb "n=20" gm/dl	0.011	0.963	NS	0.225	0.341	NS
Plt "n=20" 10^6/cmm	0.152	0.523	NS	0.026	0.914	NS
BUN "n=20" mg/dl	0.29	0.216	NS	-0.408	0.074	NS
Cr 'n=20" mg/dl	0.12	0.614	NS	-0.347	0.133	NS
Albumin "n=20" gm/dl	-0.37	0.108	NS	-0.052	0.827	NS
Na "n=20" meq/L	-0.1	0.675	NS	0.188	0.428	NS
K 'n = 20" meq/L	0.508	0.022	**S**	-0.062	0.794	NS
Ca "n=20" mg/dl	0.162	0.495	NS	0.246	0.297	NS
Po4 "n=20" mg/dl	0.34	0.143	NS	0.152	0.523	NS
Ast IU	0.422	0.064	NS	-0.103	0.667	NS
Alt IU	0.169	0.477	NS	-0.307	0.189	NS
Bil total mg/dl	0.503	0.024	**S**	-0.214	0.366	NS
INR	0.19	0.422	NBS	-0.164	0.489	NS
PTT sec	-0.065	0.786	NS	0.287	0.22	NS

* Spearman test was used

Table (18): Correlation between B2-IgM and other variables in patients with positive history of vascular access occlusion (group C) cont)

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
ESR 1st hour	0.222	0.347	NS	0.226	0.339	NS
CRP mg/dl	-0.039	0.87	NS	0.308	0.187	NS
Doppler flow ml/min n=17	0.577	0.031	**S**	-0.116	0.692	NS
B2-IgG u/ml	0.048	0.842	NS			

*Spearman test was used

Table (19): Correlation between B2IgM, B2IgG and other variables in patient with negative history of vascular access occlusion (group D)

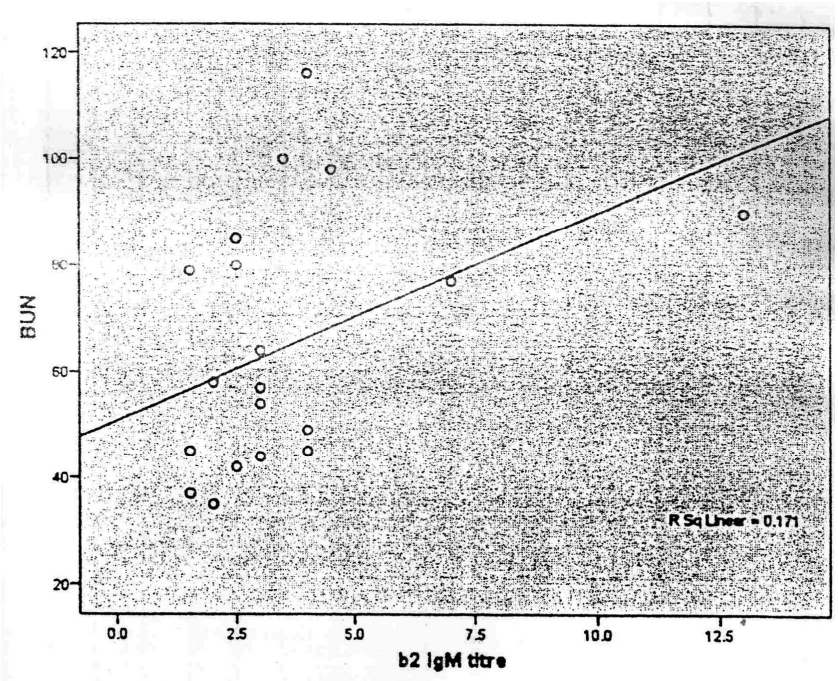
	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
Age (years) (n=20)	-0.07	0.77	NS	-0.288	0.218	NS
Duration of hemodialysis "n=30" in years	-0.304	0.193	NS	0.143	0.547	NS
Systolic BP "n=20" in mmHg	0.165	0.487	NS	0.39	0.089	NS
Diastolic BP "n = 20" in mmHg	-0.024	0.919	NS	0.204	0.388	NS
Wbc "n=20" 10^6/cmm	-0.125	0.6	NS	0.174	0.462	NS
Hb "n=20" gm/dl	0.027	0.909	NS	0.065	0.784	NS
Plt "n=20" 10^6/cmm	0.12	0.613	NS	0.104	0.662	NS
BUN "n=20" mg/dl	0.509	0.022	**S**	0.176	0.458	NS
Cr 'n=20" mg/dl	0.157	0.508	NS	-0.099	0.679	NS
Albumin "n=20" gm/dl	-0.068	0.775	NS	0.251	0.258	NS
Na "n=20" meq/L	0.28	0.232	NS	-0.35	0.131	NS
K 'n = 20" meq/L	-0.172	0.469	NS	-0.069	0.771	NS
Ca "n=20" mg/dl	0.344	0.138	NS	0.311	0.183	NS
Po4 "n=20" mg/dl	0.149	0.531	NS	0.024	0.919	NS
Ast IU	-0.025	0.917	NS	0.003	0.999	NS
Alt IU	-0.061	0.779	NS	0.297	0.203	NS
Bil total mg/dl	0.131	0.582	NS	0.406	0.076	NS
INR	0.06	0.801	NS	-0.242	0.304	NS
PTT sec	-0.088	0.712	NS	-0.341	0.141	NS

* Spearman test was used

Table (20): Correlation between B2 IgM and other variables in patient with negative history of vascular access occlusion (group D) (cont).

	B2-IgM titre u/ml			B2-IgG tire u/ml		
	*r value	*P* value	Sig.	*r value	*P* value	Sig.
ESR 1st hour	0.248	0.292	NS	-0.363	0.115	NS
CRP mg/dl	-0.104	0.662	NS	-0.207	0.381	NS
Doppler flow ml/min n=17	0.027	0.909	NS	-0.050	0.836	NS
B2-IgG u/ml	0.19	0.423	NS			

* Spearman test was used



Correlation between B2-IgM and BUN in patients with negative history of vascular access occlusion

IgG Abs, and LA with aCL IgG Abs, when considered separately, venous thrombosis was more strongly related to LA Abs, while arterial thrombosis was more closely associated with aCL (mainly IgG class) and anti-B_2-GPI IgG Abs.

Recent studies have shown a high prevalence of antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies) among patients on hemodialytic treatment (9).

Anticardiolipin antibodies represent a group of antibodies from the family of antiphospholipid antibodies. Antiphospholipid antibodies have been implicated in a variety of diseases bu the exact meaning in uremia is not understood clearly. For some scientists, the presence of anticardiolipin antibodies represent a risk factor for vascular access thrombosis(10).

The B_2-GPI induced by infections may bind to "self" aPL thus forming an immunogenic complex against which a PL are then produced (11).

aPL have also been found to be raised in a large number of infections diseases, such as syphilis, HIV infection, malaria, leprosy, and viral infections, including hepatitis C (HCV), where they are not usually associated with the clinical complications attributed to them(12).

The prevalence of aPL in infections has mainly been reported for the IgG and IgM isotypes, although a few recent studies have also investigated the IgA isotype in some infections(13).

Vascular access

Thrombosis is a major problem in hemodialysis units, resulting in increased patient morbidity, hospitalization and overall dialysis cost (14).

Despite the great prevalence of HCV among hemodialysis patients, no available study have discussed the relation of HCV and presence of Beta-2-glycoprotein I antibodies and its thrombotic complications including vascular access in hemodialysis patients.

In our study, we assessed the frequency of anti-B_2 glycoprotein I antibodies and its possible relation to thrombotic complications including vascular access dysfunction in hepatitis C seropositive hemodialysis patients in a sample of Egyptian patients on regular hemodialysis.

At present the arteriovenous fistula (AVF) is still considered to be the vascular access of choice (15).

Unfortunately, creation of an AVF is not always possible. Nowadays, polytetrafluoroethylene (PTFE) is the most used graft-material. Unfortunately arteriovenous graft (AVG) appear to be associated with a significantly higher risk of thrombosis and infection as compared with AVF (16).

Both group A (HCV+ve) and group B (HCV-ve) were similar as regard age, sex, duration of hemodialysis and type of vascular access.

Our study revealed the frequency of B_2-IgM positive or borderline was 25% (10/40 patients) [4/20 HCV +ve patients, 6/20 HCV-ve patients] while the frequency of B_2 IgG was 30% (12/40 patients) [5/20 HCV +ve patients, 7/20 HCV-ve patients].

This result was different from **Sands *et al.* (2001)**(4), who studied B_2-GP I antibodies and vascular access thrombosis in hemodialysis patients. The frequency of B_2-GPI antibodies was 10.2% (9/88 of patients).

In our study, we didn't find a significant difference in B_2-glycoprotein IgM & IgG between HCV +ve patients and HCV-ve patients. This was confirmed by the results of **Leroy *et al.*, (1998)** (17), who performed his study on chronic hepatitis C patients without renal involvement following alpha-IFN and he found similar result as ours and he found no significant difference between treated and control groups as regard B_2-glycoprotein I titre.

In HCV infection, anti B_2-GPI independent aCL are reported to be raised in 17-44% of patients, whereas raised anti-B_2-GPI and aPL are seldom found. In small cohort of HCV patients, studying all three aPL isotypes, **Gugliemone *et al.* (2001)**(18) found that 30% were positive for anti-B_2 GPI. Elevated levels of aCL were found by **Ordi-Ros *et al.* (2000)** (19), in 3.3% of patients with HCV infection and these were all beta-2 glycoprotein I (B2-GPI)-independent.

Sthoeger *et al.* (2000)(20), found elevated levels of aCL in 44% of HCV patients but once again no relationship to any APS related clinical manifestations were evident.

Dalekos *et al.* (2000)(21) found that 37.3% of their HCV patients in Northern Greece had aCL positivity.

Cacoub *et al.* (2000)(22), studying 321 patients, found aCL positivity in 27% of their patients with chronic HCV infection.

In our study, on comparing HCV positive patients and HCV negative patients as regard inflammatory markers (ESR,CRP), we didn't find a significant difference between the two groups.

This result agrees with that of **Zumrutdal *et al.* (2007)** (23), who had investigated the influence of anti-HCV positivity on markers of malnutrition and inflammation in hemodialysis (HD) patients. Serum levels of CRP and ESR, showed no statistical significant difference between HCV positive and HCV negative patients.

To our knowledge, we are the first to study the level of B_2 glycoprotein I in HCV positive prevalent hemodialysis patients and we found no relation between HCV and B_2 glycoprotein I nor fistula flow.

In our study, we assessed the possible relation of anti-B_2 glycoprotein I antibodies to thrombotic complications including vascular access.

We classified the patients according to history of vascular access occlusion into two groups each of them was 20 patients. The first group had a positive history of vascular access occlusion (group C) and the available vascular access was 14 AVF, 4 PTFE grafts and 2

catheters while the second group had negative history of vascular access occlusion (group D) and the available vascular access was 20 AVF.

In our study, elevated B_2-glycoprotein IgM titre was present in 40% (8/20 in patients with previous vascular occlusion, group C) and 10% (2/20 in patients with no previous vascular access occlusion, group D). On comparing both groups, we found significant association between recurrent vascular access thrombosis and B_2-glycoprotein IgM antibodies ($p = 0.028$).

This was in contrast to the study done by **Sands *et al.* (2001)**(4), who found elevated, B_2 – glycoprotein antibodies was present in 10.2% (9/88 patients), increased thrombotic rates were not associated with elevated anti-human B_2-GPI antibody level in PTFE.

On the other hand, elevated B_2-glycoprotein IgG tire was present in 40% (8/20) in patients with previous access occlusion and 20% (4/20) in patients with no previous access occlusion. There was no significant association between recurrent vascular access thrombosis (VAT) and elevated B_2- glycoprotein IgG antibodies.

This result was similar to that of **Sands *et al.* (2001)**(4), who studied human B_2-glycoprotein 1 (B_2 – GPI) and found no association between elevated anti-human B_2 – GPI antibody levels and increased thrombotic rates.

In our study, patients having positive B_2-glycprotein IgM antibodies had thrombotic risk more than 6 folds than negative one and most of them had AVF (6/8) patients, while (2/8) patients had graft.

This result was similar to **Sands *et al.* (2001)**(4), who found that patiens with PTEE grafts and antibodies to one or more of these proteins (prothormbin, factor V, and B_2-glycoprotein I) plasma proteins had over six fold higher thrombosis rates despite the different type of vascular access.

This difference between our results and **Sands *et al.* (2001)**(4), may be due to the predominance of AVF presence in our patients, and about 50% of our patients are HCV positive, and lastly that our patients are a different population. In addition, **Sands *et al.* (2001)**(4), studied multiple antibodies, his study included diabetic patients, smokers and collagen diseases that are excluded in our study while we studied glycoprotein I IgM, IgG antibodies as separate factor.

In our study on comparing the two groups we found no statistical difference as regards variables especially serum albumin, hemoglobin and intradialytic weight gain despite they are inerinminated in causing recurrent vascular access occlusion.

On the other hand, our results was in contrast to that of **Cheng *et al.* (2003)**(24), who found significant difference as regards serum albumin between patients with recurrent vascular access failure versus patients with longer vascular access survival, being higher in longer vascular access survival patients.

In our study, we compared ESR, CRP in patients with positive history of vascular access occlusion and patients with negative history of vascular access occlusion and we found no significant difference between the two groups.

Our results were different from that of **Che *et al.* (2009)** (25), who used Cox regression with adjustments for age, systolic blood pressure and vascular access types, serum C-reactive protein (CRP). These parameters were found to be independently associated with an increased risk for vascular access thrombosis, with a hazard ratio of 1.4 (95% confidence interval: 1.01-1.27, $p = 0.017$). High serum CRP levels was associated with the development of vascular access thrombosis in chronic HD patients.

Univariate analysis of B_2 glycoprotein I IgM titre with other variables was also assessed in our study, and we found significant negative correlation between volume of flow and B_2-IgM titre ($p = 0.03$), we concluded that elevated B_2 – GPI IgM titre is associated with decreased fistula volume of flow by Doppler .No related studies had assessed similar relations.

Conclusion:

Our study had showed significant association between recurrent vascular access thrombosis and elevated. B_2-glycoprotein IgM antibodies and negative correlation between volume of flow by Doppler and B_2-IgM titre.

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