Study of B2 glycoprotein I antibodies in hepatitis c seropositive prevalent hemodialysis patients

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Presented by

Shaimaa Zaki Abd El Megied
M.B.B.Ch

Supervised by

Prof. Dr. Mohamed Ali Ibrahim
Professor of Internal Medicine & Nephrology
Faculty of medicine, Ain Shams University

Dr. Mona Hosni Abd el Salam
Assistant professor of Internal Medicine & Nephrology
Faculty of medicine, Ain Shams University

Dr. Walid Ahmed Bichari
Lecturer of Internal Medicine & Nephrology Faculty of medicine, Ain Shams University

Ain Shams university
Faculty of Medicine
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دراسة توطئة مقدمة
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مقدمة من

ط/ شيماء زكى عبد المجيد

بكالوريوس الطب والجراحة

تحت اشراف

أ.د/ محمد على ابراهيم

استاذ الباطنة العامة والكلى
كلية الطب جامعة عين شمس

د/ منى حسنى عبد السلام

استاذ مساعد الباطنة العامة والكلى
كلية الطب جامعة عين شمس

د/ وليد احمد بشارى

مدرس الباطنة العامة والكلى
كلية الطب جامعة عين شمس

جامعة عين شمس
كلية الطب
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List of Abbreviations

ACA	anticardiolipin antibodies
acl	anticardiolipin antibodies
APC	Activated protein C
aPL	Phospholipid antibodies
apoER2	apolipoprotein E receptor 2
APS	anti-phospholipid syndrome
aPT	anti-prothrombin
AVF	arterio-venous fistula
AVG	arterio-venous grafts
B2GPI	B2-Glycoprotein I
bFV	bovine factor V
CAPS	catastrophic antiphospholipid Syndrome
CL	cardiolipin
CTLA-4	cytotoxic T lymphocyte associated antigen 4
C-X-C motif	chemokine
CXCL4	ligand 4
DOPPS	Dialysis Outcomes and Practice Patterns Study
ESRD	End stage renal disease
HBV	hepatitis B virus
HCV	Hepatitis C virus
HD	hemodialysis
HDL	High density lipoprotein
Hf II	human factor II
HITT	heparin-induced thrombocytopenia and thrombosis syndrome
HIV	human immunodeficiency virus
HO-1	Heme oxygenase-1
Ig	immunoglobulin
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M

LA	Lupus anticoagulant
LCMV	lymphocytic choriomeningitis virus
LDL	Low density lipoprotein
MAP	Mitogen-activated protein
MCII	mixed cryoglobulinemia type II
MI	myocardial infarction
MTHFR	The methylene tetrahydrofolate reductase
NAPS	nephropathy of APS
NHL	non-Hodgkin's B cell lymphomas
PAI-1	plasminogen activator of type 1
PAI-1	plasminogen activator inhibitor- 1
PAPS	primary APS
PCR	Polymerase chain reaction
PD-1	programmed cell death 1
PF4	Platelet factor 4
PGI2	prostacyclin
PLs	phospholipids
PP	Pulse pressure
PS	phosphatidylserine
PTFE	polytetrafluoroethylene
Qa	Access flow
RFLP	restriction fragment length polymorphism
SLE	systemic lupus erythematosus
SS	Sjögren's syndrome
TGF- P 1	Transforming growth factor- p 1
Tim-3	T cell immunogiobulin and mucin domain containing molecule 3
TLR-4	Toll-like receptor 4
TNF-a	Tumor necrosis factor-a
t-PA	plasminogen tissue activator
TXA2	thromboxane A2
u-PA	urokinase plasminogen activator
VSMC	vascular smooth muscle cell

Index

1	***Introduction***	***1***
2	***Aim of the study***	***4***
3	***Review***	
	Chapter 1 ***B_2- glycoprotein I***	***5***
	Chapter 2 ***The relation between B2 Glycoprotein I , anticardiolipin antibodies & thrombosis risk***	***29***
	Chapter 3 ***B2 Glycoprotein I antibodies in hemodialysis***	***48***
	Chapter 4 **B2 Glycoprotein I antibodies in HCV positive patient**	***79***
4	***Patient & methods***	***94***
5	***Result***	***100***
6	***Discussion***	***127***
7	***Summary***	***137***
8	***Conclusion***	***141***
9	***Recommendation***	***142***
10	***Appendix***	***143***
11	***References***	***155***
12	***Arabic Summary***	***-***

List of table

	Tables of Review	
*	Prognostic factors affecting vascular access patency of hemodialysis	
	Tables of Results	
1.1	comparison of quantitative variables in group (A) & group(B)	102, 103
1.2	comparison of group (A) & group(B) as regard B_2 ESR&CRP	104
1.3	comparison of group (A) & group(B) as regard B2 IgM & B2IgG	105
1.4	comparison of group (A) & group (B) as regard b2 IgM & B2 IgG Titre	106
1.5	Risk estimation via odds ratio for: B2 IgM & B2 IgG positive or borderline	107
2	Co-relation between B2-IgM ,B2-IgG and other variables in HCV +Ve (Group A)	.giSjBB?
3	Co-relation between B2-IgM, B2-IgG and other variables in HCV-Ve(Group B)	112, 113
4.1	Comparison of quantitative variables in patients with positive & negative history of vascular access occlusion.	114, 115
4.2	comparison of patients with positive & negative history of vascular access occlusion as regard ESR&CRP	116
'4.3	comparison of patients with positive & negative history of vascular access occlusion as regards B2- IgM &B2- IgG	117 s
4.4	comparison of patients with positive & negative history of vascular access occlusion as regards B2- IgM &B2- IgG titre	119
4.5	Risk estimation via odds ratio for: B2 IgM &B2 IgG	120
5	Co-relation between B2IgM, B2IgG and other variables in patients with positive history of vascular access occlusion	121, 122
6	Co-relation between B2IgM , B2IgG and other variables in patient with negative history of vascular access	124, 125
	Tables of Discussion	
*	Comparison between our study and sands et al., 2001	132

List of figures

	Figures of review	
1	B2-GPI inhibits protein c	**10**
2	Physiology of the coagulation system	**31**
3	Physiology of the fibrinolytic system	**31**
4	Coagulation cascade & phospholipid	**34**
5	Far-infrared (FIR) therapy improves access flow and unassisted patency of AVF in HD patients	**68**
6	Mechanism of action of anti-protein C antibodies	**77**
	Figures of Results	
1	Comparison of group (A) & group (B) as regard B2 lgM & B2 IgG	**106**
2	Comparison of group (A) & group(B) as regard B2 IgM & B2 IgG litre	**107**
3	Odds ratio for: B2 IgM &B2IgG positive or borderline in group A &B	**108**
4	Correlation between B2- IgM & total bilrubin in group A (HCV+ve)	**111**
5	Correlation between B2- IgG & BUN in group A (HCV+ve)	**111**
6	Correlation between B2- IgG & creatinine in group A (HCV+ve)	**111**
7	Comparison of patients with positive & negative history of vascular access occlusion as regard B2 IgM &B2IgG.	**118**
8	Comparison of patients with positive & negative history of vascular access occlusion as regards B2- IgM &B2- IgG titre titre	**119**
9	odds ratio for: B2 IgM & B2 IgG in patient with positive & negative history of vascular access occlusion	**120**
10	Correlation between B2-IgM & Doppler volume of flow in patients with positive history of vascular access occlusion	**123**
11	Correlation between B2- IgM & Total bilrubin in patients with positive history of vascular access occlusion.	**123**
12	Patients with positive history of Correlation between B2-IgM & K in vascular access occlusion.	**123**
13	Correlation between B2- IgM & BUN in patients with negative history of vascular access occlusion.	**126**

Introduction:

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

(Caronti et al, 1999).

Beta-2-glycoprotein I (beta 2GPI), is a phospholipid-binding protein eventually bound to phospholipid surfaces.

(Arnout., 2000)

B2GP1, in vitro studies suggest that it likely functions as a natural anticoagulant. B2GP1 has been shown to inhibit intrinsic pathway activation and prothrombinase activities on the surface of activated platelets and synthetic phospholipid vesicles.B2GPl also inhibits the activity of activated protein C on procoagulant surfaces. B2GP1 inhibits ADP-induced platelet aggregation and participates in the etiology of several thrombolytic diseases Plasma from normal individuals contains low concentrations of IgG autoantibodies to beta 2 GPI (beta 2 GPI antibodies) that are of moderate affinity and react with an epitope on the first domain near the N terminus. ***(Jay et al., 2010)***

The presence of anti-B2GPI antibodies can be related to the development of arterial and venous thromboses, venous thromboembolism, thrombocytopenia and fetal loss. Anti- B2GPI antibodies are found in the immunoglobulin classes IgG, IgM and IgA.

(Kandiah et al, 1995).

Sands et al., 2001 showed that hemodialysis patients had elevated anti- B2GPI antibodies. The presence of elevated antibody levels to one or more of these proteins is associated with an increased thrombotic risk.

The antiphospholipid syndrome is a disorder of hypercoagulability in association with circulating anti-phospholipid antibodies directed against epitopes on oxidized phospholipids complexed with a glycoprotein, beta2-glycoprotein I, or against the glycoprotein itself. Disorders associated with antiphospholipid antibodies but not the antiphospholipid syndrome, such as HIV and hepatitis C infection, appear to lack antibodies to beta2-glycoprotein I. Patients with systemic lupus erythematosus have a high incidence of antiphospholipid antibodies with a high risk of thrombosis,often associated with

anticardiolipin antibodies, beta2-glyocoprotein I antibodies, and the presence of the lupus anticoagulant

(Joseph et al, 2001).

Previously there are several attempts to detect anti-cardiolipin antibodies (acL) IgM, IgG in HCV positive hemodialysis patient. However the relation between its presence and thrombotic events including fistula thrombosis is 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 titer in these patients. Presence of hepatitis C may be a cofactor.

(Chuang et al., 2005)

Ozmen et al., (2009), showed that prevalence of IgG-aCL in chronic HD patients was 14.6% and no patient had a positive value of the IgM-aCL test. HCV replication was detected in 52 out of 76 patients. No significant difference in primary or secondary AVF failure was found between patients with elevated and normal aCL.

Aim of the study:

The aim of the study is to evaluate the frequency of anti-B2glycoprotein I antibodies and its possible relation to thrombotic complications including vascular access dysfunction in Hepatitis C seropositive hemodialysis patients.

B- Glycoprotein I

The first study of anti-Phospholipid antibodies began in 1906, when Wasserman introduced a serological test for Syphilis. In 1942, the active component was found to be a phospholipid, which was designated Cardiolipin. In the 1950s it became clear that a number of people had positive tests for syphilis without any evidence of the disease. This phenomenon was referred to as the biological false positive serological test for syphilis. A high prevalence of autoimmune disorders, including systemic lupus erythematosus (SLE) and Sjogrens Syndrome occurred in this group of patients.

(Ricard and Ronald .,2005)

The presence of circulating anticoagulants in patients with SLE was first documented in 1952 and was associated with increased risk of paradoxical Thrombosis in 1963. The term Lupus anticoagulant (LA), first used in 1972, is clearly a misnomer, because LA is more frequently encountered in patients without lupus and is associated with thrombosis rather than abnormal bleeding. During the last years it became clear that the optimal binding of anti-

Phospholipid antibodies is depending on a cofactor termed B2-Glycoprotein I (apolipoprotein H) (B2GPI).

(Hughes et al., 1986)

In 1990, it was found that binding of anti-Phospholipid antibodies(aPL) to phospholipid was enhanced in autoimmune conditions by a "cofactor" known as B2 glycoprotein I (B2GPI)-a glycoprotein with anticoagulant properties, whereas the non-thrombogenic aPL did not require this cofactor to enhance binding .

(Asherson et al., 2003)

Structure of b_2-Glycoprotein I:

B2-Glycoprotein I is abundantly present in plasma (~200 ug/ml) and is mainly synthesized in the liver, although mRNA coding for B2GPI has been found in a variety of cells such as trophoblasts, placental cells, endothelial cells, and neurons . The mature sequence of human b2GPI consists of 326 (44 kDa) amino acids (aa). It is composed of five repeating units that belong to the complement control protein family. The first four domains have ~60 aa residues and 4 cysteines each, with potential disulfide bridges joining the first to third and the second to

fourth cysteines to contribute to a "looped-back" structure, called Sushi domains. ***(Van et al., 2005)***

B_2-Glycoprotein I is characterized by 5 "sushi domains." The fifth sushi domain contains the binding site, which attaches to activated cellular surfaces. Lysine-rich segments are found in the fifth sushi domain and are responsible for binding to activated cellular surfaces.

(Douglas., 2002)

The fifth domain is aberrant, having 82 aa and three disulfide bridges. A positively charged (multiple lysine) region between Cys^{281} –Cys^{288} in domain V is highly conserved and a critical phospholipid-binding site. The flexible loop Ser^{311}-Lys^{317}, containing Trp^{316}, which is essential for phospholipid binding, is located in the middle of this charged region. Domain V has also been described to interact with anionic hydrophobic ligands. Domain I of b2GPI harbors another cationic region. Involvement of this region in binding to phospholipid (PL) has also been described. **(Van et al., 2005)**

Antiphospholipid antibodies bind to B_2-GPI primarily in the third and fourth sushi domains. However,