Study of the HCV status effect on soluble P- selectin levels as a marker of platelet Activation in hemodialysis patients

Thesis

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
AGEs	Advanced glycation end products
ASCVD	Atherosclerotic cardiovascular disease
ATP	Adenosine triphosphate
BM	Bone marrow
CA	Cellulose acetate
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
cGMP	Cyclic guanosine monophosphate
CIMT	Carotid intima–media thickness
CKD	Chronic kidney disease
CTA	Cellulose triacetate
CU	Cuprammonium cellulose
DIC	Differential interference contrast
ECs	Endothelial cells
EPO	Erythropoietin
ESAs	Erythropoiesis-stimulating agents
ESRD	End-stage renal disease
FGF	Fibroblast growth factor
FRs	Free radicals
HCV	Hepatitis C virus
HD	Hemodialysis
HDL	high-density lipoprotein

IL	Interleukin
LDH	lactic dehydrogenase
M-CSF	Megakaryocytes colony stimulating factors
MHD	Maintenance hemodialysis
MICS	Malnutrition-inflammation complex syndrome
MPV	Mean platelet volume
NF	Nuclear factor
NO	Nitric oxide
OCS	Open canalicular system
PAF	Platelet-activating factor
PAN	Polyacrylonitrile membrane
PBMC	Peripheral blood mononuclear cells
PDGF	Platelets derived growth factors
PES	Polyethersulfone
PF4	Platelet factor 4
PGI2	Prostacyclin
PMMA	Polymethylmethacrylate
PS	Polysulfone
PSGL-1	P-selectin glycoprotein ligand-1
P-SH	plasma sulfhydryl
PTH	Parathyroid hormone
PVP	Polyvinylpyrrolidone
ROS	Reactive oxygen species

SAA	Serum amyloid A
SOD	Superoxide dismutase
SPAN	Uncharged Polyacrylonitrile membrane
sPsel	Soluble p selectin
TF	Tissue factor
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TPO	Thrombopoietin
TSP	Thrombospondin
TXA2	Thromboxane A2

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INTRODUCTION

Early stages of chronic kidney disease (CKD) are typically associated with a prothrombotic tendency, whereas in its more advanced stage patients also suffer from a bleeding diathesis, suggesting that abnormal platelet function is a major contributor ***(van Bladel et al., 2012)***.

CKD is thus characterized by a delicate balance in which deficient hemostasis paradoxically coexists with enhanced risk of thrombosis, and in which platelet abnormalities may play an important role ***(Plé et al., 2012)***.

P-selectin (CD62) belongs to the selectin family of adhesion molecules. P-selectin is a biologically relevant molecule that is released to the surface of platelet from α granules on platelet activation and is found constitutively in a preformed state in the Weibel-Palade bodies of endothelial cells and in the α granules of platelets ***(Cox, 1998; Chen et al., 2004; Scialla et al., 2011)***. This stored P-selectin is mobilized to the cell surface within minutes in response to a variety of inflammatory or thrombogenic agents, and is involved in the adhesion of myeloid cells to activated endothelium and in the adhesion of platelets to monocytes and neutrophils **(*Chen et al., 2004)***.

Platelet P-selectin expression is increased during haemodialysis in association with increased platelet-leucocyte aggregation ***(Ashman et al., 2003)***. Moreover, the soluble form is released into plasma

during platelet activation independent of hemodialysis procedure ***(Özkan and Ulusoy, 2013)***.

Hepatitis C virus (HCV) affects a large percentage of hemodialysis patients and leads to chronic liver disease. This may further aggravate their haemostatic abnormalities ***(Pawlak et al., 2008)***. Reductions of platelet counts were more frequent in patients on dialysis particularly in HCV-positive patients. The incidence of thrombocytopenia is around 55% of HCV-positive hemodialysis patients ***(Ando et al., 2011)***. Thrombocytopenia and platelet activation manifested by increased expression of P-selectin coexisted in chronic liver disease, especially in patients with chronic hepatitis C ***(Omran et al., 2011)***.

Erythropoiesis-stimulating agents (ESAs) are a widely used treatment approach for anemia of CKD patients and prescribed worldwide since many years ***(Vecchio and Locatelli., 2012)***.The use of erythropoietin (EPO) in CKD can enhance blood coagulation by stimulating production of E-selectin and P-selectin ***(Vaziri and Zhou, 2009a; Ribeiro et al., 2013)***.

A single intravenous dose of EPO increases platelet counts as well as, circulating E-selectin and P-selectin concentrations which are responsible for the increased platelet reactivity and aggregation in healthy volunteers ***(Heinisch et al., 2012)***. The use of EPO in chronic HCV patient not only significantly increases hemoglobin levels but may also increase platelet count and activation ***(Yu et al., 2008)***.

P- selectin promotes a procoagulant state that may play a role in vascular access failure in hemodialysis patients ***(Yousry et al., 2006)***.

There is evidence for a positive correlation between venous thrombosis, atherosclerotic cardiovascular disease (ASCVD) events and soluble P-selectin (sPsel) levels ***(Ramacciotti et al., 2011; Scialla et al.,2011)***.

AIM OF the WORK

This aim of this study was to evaluate the effect of hepatitis C virus (HCV) infection on soluble P-selectin (sPsel) levels as a marker of platelet activation in chronic hemodialysis patient and receiving erythropoiesis-stimulating agents (ESAs).

OVERVIEW OF PLATELETS AND THROMBOPOIESIS

An adequate supply of platelets is essential to repair the minute vascular damage that occurs with daily life, and to initiate thrombus formation in the event of overt vascular jury. Accumulating evidence also indicates vital roles for platelets in wound repair, the innate immune response, and metastatic tumor cell biology. The average platelet count in humans ranges from 150×10^9 to 400×10^9 per liter, although the level for any individual is maintained within fairly narrow limits from day to day. While 150×10^9 to 400×10^9 per liter is considered "normal," the values derived from the mean ± 2 SDs of a group of "healthy" individuals, epidemiological evidence indicates that individuals who display platelet counts in the highest quartile of the normal range have a 2-fold increased risk of adverse cardiovascular events ***(Thaulow et al., 1991)***, and, in both experimental animal models of metastatic cancer and patients with tumors, higher platelet levels carry an unfavorable prognosis ***(Gupta et al., 2004)***.

With a lifespan of approximately 10 days, a blood volume of 5 liters, and one-third of platelets pooled in the spleen, the average adult must produce each day approximately 1×10^{11} platelets to maintain a normal platelet count under steady-state conditions, a level of production that can increase more than 10-fold under conditions of increased demand. The primary regulator of platelet production is thrombopoietin, an acidic glycoprotein produced primarily in the liver, kidney, and BM. The biochemistry and structure-activity relationships

of thrombopoietin have been carefully evaluated, as have the binding sites to its receptor, the product of the cellular proto-oncogene c-Mpl ***(Kato et al., 1998)***.

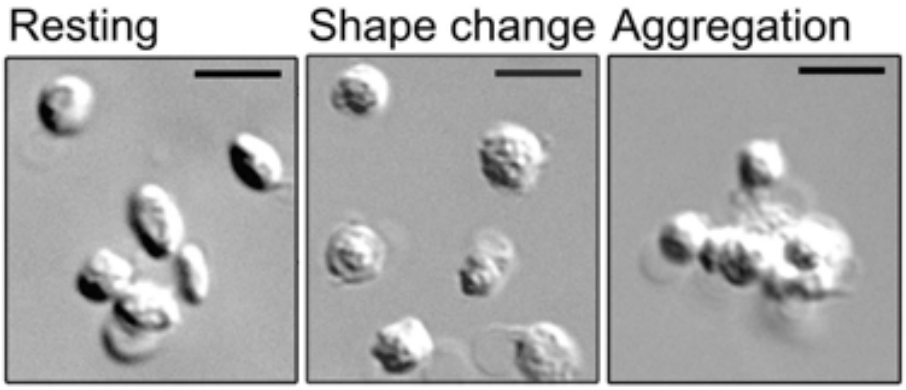

Fig. (1) Different stages of activation. DIC microscopy pictures of human platelets in three stages. the un stimulated platelets, platelets undergoing shape change and platelet aggregation ***(Gunnarsson, 2009)***.

Mean Platelet Volume And Platelet Structure at rest

In physiological conditions, platelets circulate in a resting state, protected from activation by inhibitory mediators, such as nitric oxide (NO) and prostacyclin (PGI2) released from intact endothelial cells (ECs) ***(Margetic, 2012)***.

Platelets normally do not physically interact with vascular ECs, circulating platelets are discoid in shape, with dimensions of approximately 2.0–4.0 by 0.5 μm, and a mean volume of 7–11 fl. Their shape and small size enables the platelets to be pushed to the edge of vessels, placing them in the optimum location to constantly survey the integrity of the vasculature ***(Sharathkumar and Shapiro, 2008).***

In resting platelets, granules are situated close to the Open canalicular system(OCS) membranes. Platelets have two major recognized storage granules: α and dense granules. The most abundant are α granules (about 40 per platelet), which contain proteins essential for platelet adhesion during vascular repair. These granules are typically 200 to 500 nm in diameter and are spherical in shape with dark central cores, The surface of the platelet plasma membrane at rest appears featureless in most micrographs, plasma membrane is smooth ***(Italiano et al., 2008)***.

Main platelet volume(MPV) gives information about platelet production in bone marrow. When platelets decrease in number, bone marrow megakaryocytes are stimulated by thrombopoietin, and their nucleus becomes hyperlobulated, with much higher deoxyribonucleic acid content. These stimulated megakaryocytes produce larger platelets. Thus, platelets with a higher MPV are expected to be seen in destructive thrombocytopenia when megakaryocytic stimulation is present. Conversely, platelets with a lower MPV are expected in thrombocytopenic states associated with marrow hypoplasia or aplasia. There is an inverse relationship between platelet count and MPV, resulting in a roughly constant circulating platelet mass ***(Beyan, 2012; Karaman et al., 2013)***.

Mean Platelet Volume And Structure of the Activated Platelet

Numerous factors promote platelet activation during inflammatory response. Such, EC dysfunction leads to increased platelet reactivity due to its elevated production of TXA2 and vWF

and decreased production of PGI2. Upon vessel wall injury platelets rapidly adhere to the exposed subendothelial matrix. This process is mediated by several cellular receptors as well as various adhesive and regulatory proteins present on platelets or ECs thus allowing platelet-vessel wall interaction and subsequent thrombus formation. Additionally, in the setting of inflammation induced activation of haemostasis, platelets can be directly activated with inflammatory mediators such as proinflammatory cytokines or platelet-activating factor (PAF) in case of both non-infectious and infectious infammatory states or with endotoxin in case of sepsis. Further, thrombin generated by activated coagulation cascade is one of the strongest platelet activators ***(Margetic, 2012)***.

Platelets activated, in response to inflammation, vascular damage, variety of agonists Platelet surface membrane invaginates to form a tubular network, the canalicular system provides a conduit for the discharge of the granule content following platelet activation ***(Craig et al., 2010)***.

Platelet undergo rapid and dramatic changes in cell shape, up regulate the expression and ligand -binding activity of adhesion receptors, and secrete the contents of their storage granules after the granules fuse and exocytose into the OCS ***(Italiano et al., 2008)***.

Activated platelets will bind to the wall of inflamed microvessels by attaching either directly to ECs or to leucocytes that are already adherent on the vessel wall ***(Stokes and Granger, 2012)***.

Mean platelet volume is an indicator of platelet activation, which has an important role in the pathophysiology of thrombosis.