C-REACTIVE PROTEIN, A POSSIBLE VALUABLE PREDICTIVE INFLAMMATORY MARKER IN HCV POSITIVE HEMODIALYSIS PATIENTS

Thesis

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List of Abbreviations

ACE	Angiotensin converting enzyme
AGEs	Advanced glycation end products
ANG-II	Angiotensin II
ASA	Acetyl Salicylic Acid
AT1-R	Angiotensin Type 1 Receptor
ATP	Adenosine triphosphate
BMI	Body mass index
CAD	Coronary Artery Disease
CCR5	C-C chemokine receptor type 5
CKD	CHRONIC KIDNEY DISEASE
CVD	Cardiovascular disease
DM	DIABETES MELLITUS
DNA	Deoxyribonucleic acid
eNOS	Endothelial Nitric Oxide Synthetase
EPCs	Endothelial Progenitor Cells
ESR	Erythrocyte sedimentation rate
ESRD	End stage renal diease
ET-1	Endothelin-1
GFR	Glomerular filtration rate
GH	Growth hormone
HCV	Hepatitis C virus
HD	Hemodialysis
HMG CoA	The 3-Hydroxy-3-Methylglutaryl-Coenzyme A
ICAM-1	Intercellular adhesion molecule 1
IGF-1	Insulin-like growth factor 1
IL-1	Interleukin 1
IL-10	Interleukin 10
IL-6	Interleukin 6

List of Abbreviations (Cont…)

LDL	Low density lipoprtien
LVH	left ventricular hypertrophy
MAP	Mitogen-activated protein
MCP-1	Monocyte Chemotactic Protein-1
MDRD	Modification of Diet in Renal Disease
MI	Myocardial Infarction
MIA	The malnutrition, inflammation, and atherosclerosis
OPG	Osteoprotegerin
PAI-1	Plasminogen Activator Inhibitor-1
PD	Peritoneal dialysis
PEW	Protein-energy-wasting
PTX3	Pentraxin-related protein
ROS	Reactive Oxygen Species
RRF	Residual renal function
SAP	Serum amyloid P component
sCD163	Soluble haemoglobin scavenger receptor
SLE	Systemic lupus erythematosus
TNF	Tumor necrosis factor
TWEAK	Soluble tumor necrosis factor-like weak inducer of apoptosis
USA	United states of America
VCAM-1	Vascular cell adhesion protein 1
VSM	Vascular Smooth Muscle

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INTRODUCTION

C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation (i.e. C-reactive protein is an acute-phase protein). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex **(Thompson et al., 1999).**

CRP and other acute phase proteins are elevated in dialysis patients and cardiovascular diseases represent the single largest cause of mortality in chronic renal failure patients **(Panichi et al., 2001).**

As CRP is so strongly associated with vascular disease, it has been suggested that this protein is not only a marker, but also a mediator, of atherogenesis. Indeed, recent in vitro data from studies on endothelial cells, monocytes-macrophages and smooth muscle cells support a direct role for CRP in atherogenesis. In ESRD, hs-CRP has been proven to be a strong predictor of both cardiovascular and all-cause mortality, and associated with oxidative stress, vascular calcification and endothelial dysfunction (**Stenvinkel and Lindholm, 2005).**

Hepatitis C virus (HCV) infection is a major health problem in patients with end-stage renal disease (ESRD). The incidence of

acute HCV infection during maintenance dialysis is much higher than that in the general population because of the risk of nosocomial transmission. Following acute HCV infection, most patients develop chronic HCV infection, and a significant proportion develops chronic hepatitis, cirrhosis, and even hepatocellular carcinoma. Overall, chronic hepatitis C patients on hemodialysis bear an increased risk of liver-related morbidity and mortality, either during dialysis or after renal transplantation **(Liu and Kao, 2011)**.

AIM OF THE WORK

The aim of this work is to study the response of HCV positive HD Patients and its impact on C-reactive protein level as a surrogate marker of inflammation.

HISTORY AND NOMENCLATURE

C-reactive protein was discovered in Oswald Avery's laboratory during the course of studies of patients with streptococcus pneumoniae infection **(Tillet and Francis, 1930)**.

Sera obtained from these patients during the early acute phase of the illness were found to contain a protein that could precipitate the "C" polysaccharide derived from the pneumococcal cell wall. Although phosphocholine was the first defined ligand for C-reactive protein, a number of other ligands have since been identified **(Black et al., 2004)**.

Genetics:

The CRP gene is, located on the short arm of the first chromosome (1q21-q23). Baseline levels of CRP show a clear heritability about 40% in studies of families **(Szalai et al., 2002)**. Currently, three polymorphism in CRP gene that are associated with changes in CRP level have been documented **(Brull et al., 2003)**.

By identifying genetic variations in the CRP gene, at-risk genotypes may be deciphered, providing additional information for overall risk assessment. Such genotype specific risk categories may identify individuals who have relatively low serum CRP levels yet display an enhanced proinflammatory phenotype, enhanced endothelial cell activation and cardiovascular risk **(Khreiss et al., 2004)**.

Biochemistry:

CRP is a 224-residue protein with a monomer molar mass of 25106 Daltons. The protein is an annular pentameric disc in shape. Proteins with this type of configuration are known as pentraxins **(Hirschfield and Pepys, 2003)**.

The pentraxin family, named for its electron micrographic appearance from the Greek penta (five) and ragos (berries) comprises CRP and Serum Amyloid P component (SAP) in man **(Pepys and Hirschfield, 2003)**.

Sites of Synthesis of CRP:

CRP induction is a part of a larger picture of liver gene expression during the inflammatory states,Synthesis of CRP principally in hepatocytes is regulated at the transcriptional level under the control of a cascade of cytokines, including Interleukin-1 (IL-1), TNF and IL-6, originating at the site of pathology **(Gabay and Kushner., 1999)**.

Extrahepatic synthesis of CRP has also been reported in neurons, atherosclerotic plaques,monocytes,and lymphocytes. The mechanisms regulating synthesis at these sites are unknown, and it is unlikely that they substantially influence plasma levels of CRP **(Jialal et al., 2004)**.

Ligand binding and biological role of CRP:

Human C-reactive protein is a calcium-dependent ligand binding protein, which binds with highest affinity to phosphocholine

residues as well as a variety of other autologous and extrinsic ligands and aggregates or precipitates the cellular, particulate or molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins damaged cell membranes,a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles **(Pepys et al., 1994)** and apoptotic cells **(Gershov et al., 2000)**.

Extrinsic ligands include many glycan,phospholipids and other components of micro-organisms, such as capsular and somatic components of bacteria, fungi and parasites,as well as plant products **(Du Clos, 2000)**.

When CRP is aggregated or bound to macromolecular ligands, human CRP is recognized by C1q and potently activates the classical complement pathway, engaging C_3, the main adhesion molecule of the complement system, and the terminal membrane attack complex, C_5-C_9 **(Mold et al., 1999)**.

Bound CRP may also provide secondary binding sites for factor H and thereby regulate alternative-pathway amplification and C_5 convertases **(Du Clos, 2000)**.

The secondary effects of C-reactive protein that follow ligand binding resemble some of the key properties of antibodies, suggesting that under various circumstances C-reactive protein may contribute to host defence against infection, function as a pro-inflammatory mediator, and participate in physiological and

pathophysiological handling of autologous constituents **(Hirschfield and Pepys, 2003)**.

CRP is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections **(Pradhan et al., 2001)**.

CRP is a part of the innate immunity that activates classical complement pathway after binding to ligands **(Du Clos., 2000)**.

CRP also binds to phospholipids of damaged cells, with subsequent limited activation of the complement system and enhanced uptake of these cells by macrophages **(Mold et al., 1999)**.

CRP was shown to possess proatherogenic properties. For example, CRP activates endothelial cells to express adhesion molecules, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, selectins, and the chemokine, monocyte chemotactic protein-1 **(Pasceri et al., 2001)**.

CRP also induces the secretion of interleukin-6 and endothelin-1 and decreases the expression and bioavailability of endothelial nitric oxide synthetase in human endothelial cells **(Verma et al., 2002)**. Furthermore,CRP activates macrophages to express cytokine and tissue factor and enhances the uptake of LDL **(Venugopal et al., 2002)**.

CRP also amplifies the proinflammatory effects of several other mediators, including endotoxins **(Yeh et al., 2001)**.

C-Reactive Protein concentration in health and disease:

Normal reference ranges for blood tests are less than 5-6 mg/L. In healthy volunteer blood donors, the median concentration of C-reactive protein is 0.8 mg/L **(Shine et al., 1981)**.

In the general healthy population, the median base line value is slightly higher and tends to increase with age. The women, especially older women, on average appear to have slightly higher normal values as compared to men. There is no significant seasonal variation in base-line CRP concentration **(Ford, 1999)**.

In view of the sensitivity, speed, and range of the CRP response, subjects in the general population tend to have stable CRP concentrations characteristic for each individual, apart from occasional spikes presumably related to minor or subclinical infections, inflammation, or trauma **(Hutchinson et al., 2000)**.

CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level

is mainly determined by the rate of production and hence the severity of the precipitating causes **(Dehghan, 2007)**.

The oral contraceptive use and systemic, but not transdermal, postmenopausal hormone replacement therapy are also associated with significantly raised base-line CRP concentrations without any sign of tissue-damaging inflammation **(Peters et al., 2001)**.

Overweight, obese and diabetic individuals typically have elevated serum CRP and elevated CRP levels have been found to predict the development of type 2 diabetes **(Pradhan et al., 2001)**. Consistent with this is the observation that individuals with insulin resistance syndrome typically exhibit elevated CRP levels **(Marques Vidal et al., 2002)**.

Living in an environment where air pollution is high can raise CRP values **(Peters et al., 2001)**.

It is well known that smoking elevates serum CRP. Also, some studies have shown that patients with atrial fibrillation have higher CRP levels than normal **(Chung et al., 2001)**.

Cytokines responsible for acute-phase protein production also are known to play significant roles in neuropsychologic function and dysfunction. So it is possible that detecting CRP elevation in some individuals is merely an indirect way of detecting depression **(Miller et al., 2002)**.