Cadherin 5 and Annexin V as circulating endothelial microparticles:

Prognostic markers for atherosclerotic vascular lesions in patients with chronic renal disease.

Thesis

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ABSTRACT

Background: Endothelial dysfunction has been regarded to as an early stage in the atherosclerotic process *and could be evaluated by invasive methods (as catheterization) and non invasive methods (as endothelial microparticles as Cadherin 5 and Annexin V).*

Aim of the work: This study tries to identify circulating endothelial MPs (such as Cadherin 5 and Annexin V) as potential new risk factors in the occurance of cardiovascular events in patients with chronic renal diseases.

Subjects and Methods: Cadherin 5 and Annexin V were measured in 20 healthy control, 25 patients of chronic renal disease without ischemic cardiovascular complications (Group I) and 35 patients of renal kidney disease with ischemic cardiovascular complications (Group II) by the quantitative sandwich ELISA technique.

Results: Serum **Cadherin 5** was 31.69 ± 11.23 ng/ml in group I and 86.99 ± 21.51 in group II with **highly statistical significant** difference to control group (2.63 ± 1.47) {p < 0.01}.Also group II showed **highly** statistical significant difference when compared to group I { p < 0.01 } . **Cadherin 5** recorded a high specificity (99.96%) and sensitivity (97%) at cut off 46.8 and area under the curve was 0.998. Serum **Annexin V** was 27.26 ± 11.87 ng/ml in group I and 83.73 ± 22.64 in group II with **highly statistical significant** difference to control group (0.47 ± 0.36) {p < 0.01}. Also group II showed **highly** statistical significant difference when compared to group I { p < 0.01 } . **Annexin V** recorded a high specificity (99.88%) and sensitivity (94.3 %) at cut off 39.15 and area under the curve was 0.993. *Cadherin 5 and Annexin V are more sensitive than C-reactive protein* (at cut off 36.0 sensetivity was 71.4 and specificity was 99.6) . A significant direct correlation was found between levels of Cadherin 5 and Annexin V .An inverse correlation was found between Cadherin 5 and Annexin V in one hand and ejection fraction in the other hand in patient group. A significant direct correlation was found between levels of Cadherin 5 and Annexin V in one hand and CRP & ESR in the other hand .

Conclusion: Serum Cadherin 5 and Annexin V is elevated in patients with chronic kidney disease and is considered nontraditional risk factors for prediction of cardiovascular complications especially atherosclerotic ischemic heart disease thus permitting a new therapeutic strategies of cardiovascular complications in patients with chronic kidney disease .

Key words: Cadherin 5, Annexin V , endothelial dysfunction ,endothelial microparticles , Chronic renal disease and ischemic heart disease.

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Mahmoud Farok Moghazy

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List of abbreviations

ABPM : Ambulatory blood pressure monitoring .

ACE : Angiotensin converting enzyme.

ACS : Acute coronary syndromes .

ADMA : Asymmetric dimethylarginine

AGEs : Advanced glycation end products .

Alb. : Albumin .

Alp : Alkaline phosphatase

Ang II : Angiotensin II .

ANXA5 : Annexin A 5 .

ANOVA : Analysis of variance .

ARSC : Atherosclerosis risk in community .

ATI : Angiotensin-converting enzyme Inhibitor.

AT I-R : Angiotensin 1- Receptor.

AT II-R : Angiotensin 2- Receptor .

ASO : Antistreptolysin O

ATP : Adenosine triphosphate .

AV : Arteriovenous .

BFU-E : Burst forming unit – Eryythrocyte .

BMI : Body mass index .

BP : Blood pressure.

C3 : Complement 3

C4 : Complement 4

Ca : Calcium .

CAC : Coronary artery calcification.

CAD : Coronary artery disease.

CD : Cluster differentiation.

CFU-E : Colony forming unit – Erythrocyte**.**

CHD : Coronary heart disease .

CK : Creatinine kinase .

CKD : Chronic kidney disease .

CHS : Cardiovascular health study .

cm : Centimeter .

CMV : Cytomegalovirus .

CPBI : Cephalopondin I.

CRD : Chronic renal disease .

CRI : Chronic renal impairment .

CRF : Chronic renal failure

CRP : C reactive protein.

CT : Computer tomography**.**

CV : Cardiovascular **.**

CVC : Cardiovascular complication

CVD : Cardiovascular disease.

DBP : Diastolic blood pressure .

DDAH : Dimethyle argenine dimethylargenine hydrolase .

DIC : Dissiminated intravascular coagulation .

DM : Diabetes mellitus .

DOD : Duration of dialysis .

EC : Extracellular .

ECG : Electrocardiogram.

EDD : End diastolic diameter .

EF : Ejection fraction .

e.g. : For example .

e GFR : Estimated glomerular fitration rate .

EGFR : Epidermal growth factor receptor .

EIA : Enzyme immunoassay .

ELISA : Enzyme linked immunosorbent assay .

EMP : Endothelial microparticles .

EPO : Erythropoietin .

ESD : End systolic diameter .

ESRD : End stage renal disease .

ESRF : End stage renal failure .

ET 1 : Endothelin 1

F : Female .

FCR : Fragment crystalisation receptor.

FGF : Fibroblast growth factor .

FS : Fractional shortening .

GFR : Glomerular filtration rate .

H2O2 : Hydrogen peroxide.

Hb : Haemoglobin .

HD : Hemodialysis .

HOT : Hypertension optimal treatment .

HPTH : Hyperparathyroidism.

HS : High significant .

HTN : Hypertension .

ICAM-1 : Intracellular adhesion molecule-1

IE : Infective endocarditis .

i.e. : That is to say .

IgA : Immunoglobulin A.

IgG : Immunoglobulin G.

IgM : Immunoglobulin M .

IHD : Ischemic heart disease .

IL : Interleukin .

IVP : Intravenous pyelography .

IVS : Interventricular septum .

IVST : Interventricular septum thickness.

IHD : Ischemic heart disease .

FCR : Fractional catabolic rate .

FS : Fractional shortening

g/d : Gram per dicilitre .

HD : Heamodialysis.

HDL : High-density lipoprotein

HIV : Human immunodeficiency virus .

HRP : Horse reddish peroxidase .

HRS : Hepatorenal syndrome .

HS : High significant .

hs-CRP : High sensitivity C reactive protein .

K : Potassium .

Kg : Kilogram .

KT/V : K: Dialysis clearence of urea . T : Dialysis time . V : Pateint total body water .

LL : Lower limb .

Lp(a) : Lipoprotein (a)

LPC : Lysophoshatidyle choline .

LV : Left ventricle .

LVH : Left ventricular hypertrophy.

LVM : Left ventricular mass .

LVPWT : Left ventricular posterior wall thickness.

M : Male .

MA : Microalbumiuria .

MI : Myocardial infarction .

mg : Milligram .

mmHg : Millimeter mercury .

MMP : Metalloproteinase.

Mosm/kg : milliosmol per kilogram .

MP: Microparticles .

MPO : Myeloperoxidase.

MRFIT : Multiple risk factor intervention .

m RNA : messenger ribonucleic acid.

MRI : Magnetic resonance imaging .

MTHRF : Methylene tetrahydrofolate reductase .

n : Number

Na : Sodium .

Na Cl : Sodium Chloride

NHD : Nocturnal hemodialysis .

NKF : National kidney foundation .

NO : Nitric Oxide

NOS : Nitric oxide synthase .

nPCR : normalized protein catabolic rate .

NS : Non significant .

oxLDL : Oxidized low density lipoprotein .

P: Probability of chance .

PAF : Platelete activating factor .

PAP I : Placental anticoagulant protein I .

PC : Phoshatidyle choline.

PCD : Programmed cell death

PCNA : Proliferating nuclear cell antigen.

PD : Peritoneal dialysis .

PH : Pulmonary hypertension .

PO4 : Phosphorus .

PS : Phosphatidyle serine.

PTH : Parathyroid hormone

PWT : Posterior wall thickness .

RBCs : Red blood cells.

RBS : Random blood sugar .

ROC : Receiver operating characteristic .

RTT : Renal transplantation therapy .

SBP : Systolic blood pressure .

SD : Standard deviation .

SGOT : Serum Glutamic-Oxaloacetic transaminase .

SGPT : Serum Glutamic-pyruvic transaminase .

SDMA : Symmetric dimethyl argenine.

SLE : Systemic lupus erythromatosis .

SG : Specific gravity .

SWMA : Superior wall motion abnormality .

TC : Total cholesterol.

TEE : Transesophageal Echocardiography .

TF : Tissue factor .

TG : Triglycerides .

TGF-alpha : Tumour growth factor – alpha .

TMB : Tetra methyl benzidine .

TNF $-\alpha$ **:** Tumor necrosis factor – alpha .

UACR : Urinary albumin to creatinine ratio

UEA : Urinary excretion of albumin

USRD : United States Renal Data **.**

VAC-a : Vascular anticoagulant alpha .

VCAM-1: Vascular cell adhesion molecule - 1

VE-Cadherin : Vascular endothelial Cadherin.

VSMC : Vascular smooth muscle cell .

v WF : Von willebrand factor .

INTRODUCTION

Introduction

In chronic kidney disease patients it was found that cardiovascular disease is the leading cause of death . These developments call for the development of technologies that detect subclinical cardiovascular pathology before catastrophes, such as myocardial infarction, heart failure and/or stroke occur **(Edward et al., 2008) .**

Chronic kidney disease (CKD) is associated with increased morbidity and mortality in cardiovascular disease (CVD). Apart from the tradional risk factors(such as age , gender and smoking) ; chronic inflammation, oxidative stress and endothelial dysfunction are important in CVD development in renal patients **(Annuk et al., 2005).** Even mildly impaired renal function is associated with cardiovascular complications. There are indications that endothelial dysfunction and/or chronic inflammation, which play an important role in atherothrombosis, are present even in early stages of renal insufficiency **(Stam et al., 2006).**

Endothelial dysfunction has been regarded to as an early stage in the atherosclerotic process **(Suzuki et al., 2004)** and has predictive value for ischemic events **(Migliacci et al., 2007).** Some plasma biomarkers of inflammation and endothelial dysfunction have been recently recognized as important cardiovascular risk factors **(Zoppini et al., 2006).**

Cadherin 5, also called **CD 144** or **VE- Cadherin**, is a 140 k Dalton protein belonging to the Cadherin family of cell adhesion molecules **(Takeichi ., 1990) .** Cadherin 5 is a Ca^{++} dependent cell adhesion molecule and is expressed in sclerotic lesions and is associcated with neovascularization **(Soeki et al .,2004).**

Cadherin 5 is useful as a specific marker of endothelial cell (EC) dysfunction and is useful in identifying diabetes mellitus patients with increased risk of coronary artery disease (CAD) **(Preston et al., 2003) .**

Cadherin 5 is a major determinants of cardiovascular risk in patients with end-stage renal failure (ESRF) (**Leroyer et al., 2005) .**

Another biomarker; Annexin V also called CD131 or ANXA5 is a calcium binding protein of 36 kDa which is widely present in various cells and tissues **(Kaneko et al., 1996)**.

Annexin V have been shown to be increased in patients with severe kidney failure undergoing hemodialysis and to be correlated with endothelial dysfunction and arterial stiffness **(Amabile et al. *2005*).**