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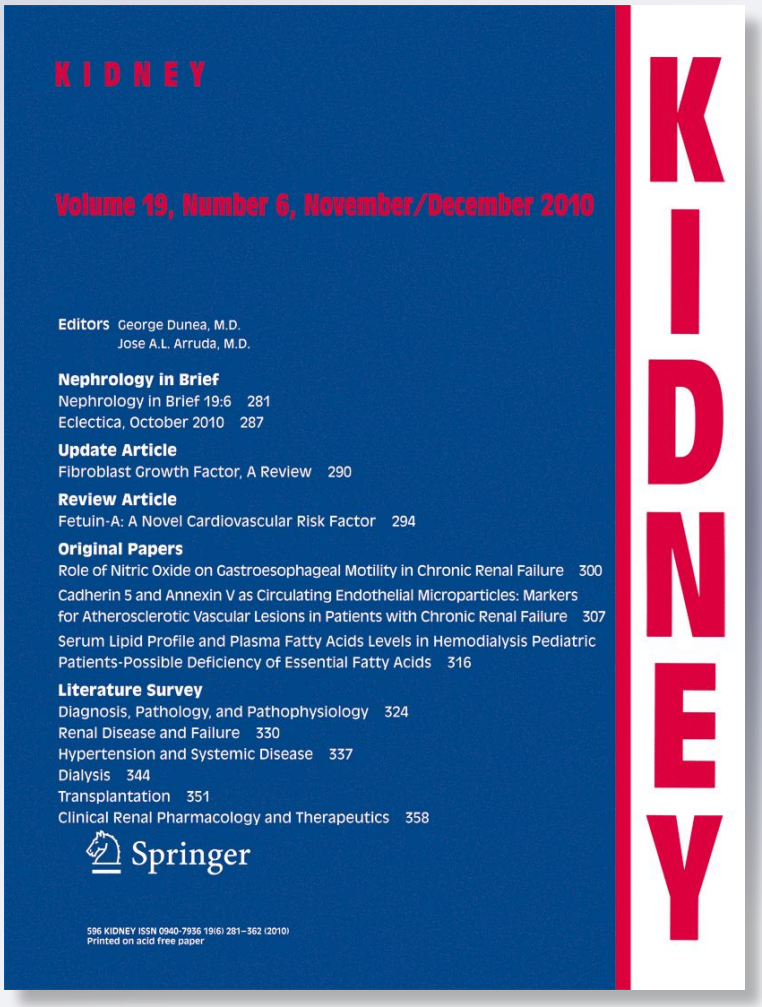

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Cadherin 5 and Annexin V as Circulating Endothelial Microparticles: Markers for Atherosclerotic Vascular Lesions in Patients with Chronic Renal Failure

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Abstract Cardiovascular disease is the leading cause of death in Chronic kidney disease patients. This study tries to identify circulating endothelial microparticles {MPs} [such as Cadherin 5 and Anexin V] in CKD patients with and without IHD as potential new risk factors of atherosclerotic vascular disease. This study was carried out in Theodor Bilharz Research Institute [TBRI] on 60 patients with chronic kidney disease on maintenance hemodialysis. They were 41 male and 19 females selected from hemodialysis unit in TBRI. They were further subclassified into the following two groups according to the Echocardiography and Electrocardiogram (ECG) to 25 patients of chronic kidney disease without cardiac complications (17 males, 8 females and ages were 53.5±9.9 years) and 35 patients of chronic kidney disease with cardiac complications (24 males, 11 females and ages were 57.5±7.4 years). Twenty healthy subjects were selected as healthy control, their age 50±9 years. Cadherin 5 & Annexin V Were done by enzyme linked immunosorbant assay (ELISA). The mean cadherin 5 levels in CKD with ischemic HD, CKD without ischemic HD and control group were 86.99±21.51, 33.21±8.65 and 2.63±1.47 respectively which significantly higher in CKD with ischemic HD and CKD without ischemic HD than control group ($p<0.01$) and significantly higher in CKD with ischemic HD than CKD without ischemic HD ($p<0.01$). As regard to the mean annexin v levels in CKD with ischemic HD, CKD without ischemic HD and control group were 83.73±22.64, 28.51±9.73 and 0.47±0.36 respectively which significantly higher in CKD with ischemic HD and CKD without ischemic HD than control group ($p<0.01$) and significantly higher in CKD with ischemic HD than CKD without ischemic HD ($p<0.01$). Endothelial dysfunction leading to atherosclerotic vascular disease in patients with CKD can be assessed quantitatively by measurement of plasma levels of endothelial microparticles such as CD144-EMP (Cadherin 5) and Annexin V.

Keywords Endothelial dysfunction · CKD · Haemodialysis · Ischemic heart disease · Cadherin5 · Annexin V

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Introduction

Cardiovascular disease is the leading cause of death in Chronic kidney disease patients. 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 [1].

Chronic kidney disease (CKD) is associated with increased morbidity and mortality in cardiovascular disease (CVD). Apart from the traditional risk factors; chronic inflammation, oxidative stress, malnutrition and endothelial dysfunction are important in CVD development in renal patients [2]. 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 in early stages of renal insufficiency [3].

Endothelial dysfunction has been regarded to as an early stage in the atherosclerotic process [4] and has predictive value for ischemic events [5]. Some plasma biomarkers of inflammation and endothelial dysfunction have been recently recognized as important cardiovascular risk factors [6].

CD 144, also called Cadherin 5 or VE—Cadherin, is a 140 k Dalton protein belonging to the Cadherin family of cell adhesion molecules [7]. Vascular endothelial (VE)—Cadherin a Ca^{++} dependent cell adhesion molecule, is expressed in other sclerotic lesions and is associated with neovascularization [8]. CD 144 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).[9]

Moreover, endothelial function may be decisively influenced by the degree of endothelial cell apoptosis. It has been revealed that increased apoptotic mircroparticle counts predict severe endothelial dysfunction independent of classical risk factors such as hypertension, hypercholesterolemia, smoking, diabetes, age or sex [10].

Annexin V is a calcium binding protein which is widely present in various cells and tissues [11].

Annexin V (also called Annexin 5, ANXA5 or CD 131) is a protein of 317 amino acids and molecular weight of 36 kDa, previously known as placental anticoagulant protein I (PAP I), vascular anticoagulant alpha (VAC-a), and calphobindin I (CPBI). It had been originally purified from anticoagulant fraction derived from human umbilical cord arteries and placenta, a rich source of Annexin V [12].

Measurement of plasma Annexin V by ELISA in the early detection of acute myocardial infarction showed elevated levels at the time of initial blood drawing, 3 h, 2 h and even 1.5 h after onset of pain [11].

Circulating CD31/ Annexin V apoptotic microparticles highly significantly predict the degree of endothelial dysfunction in humans with CAD. At present it remains unclear whether increased numbers of CD31 /Annexin V apoptotic microparticles in patients with severe coronary endothelial dysfunction are increased because of a mechanical or biochemical injury or whether endothelial cells try to eliminate noxious agents, which themselves cause the dysfunction of the vascular endothelium [10].

Aim of the Work

This study tries to identify circulating endothelial MPs (such as Cadherin 5 and Annexin V) in CKD patients with and without IHD as potential new risk factors of atherosclerotic vascular disease.

Subject and Methods

Subjects

This study was carried out in Theodor Bilharz Research Institute (TBRI).

The following groups were included in the present study:

Patient Group

We included in this study 60 patients with chronic kidney disease on maintenance hemodialysis. They were 41 male and 19 females selected from hemodialysis unit in TBRI. They were further subclassified according to the Echocardiography and Electrocardiogram (ECG) into the following two groups:

1- Group I: Included 25 patients of chronic kidney disease without cardiac complications.
   There were 17 males, 8 females and ages ranged from 44 to 63 years.
2- Group II: Included 35 patients of chronic kidney disease with cardiac complications.
   There were; 24 males, 11 females and ages ranged from 50 to 64 years.

Control Group

Twenty healthy age and sex matched subjects were selected as the control group, their age ranged from 41 to 59 years.

Exclusion Criteria:

– Any other type of inflammatory condition or systemic sepsis.
– Any other major systemic disease.

Methods

All patients and controls in this study were subjected to following:

History Taking

Laying stress on symptoms of cardiac complications (such as chest pain and dypsnea).

Clinical Examination

To confirm the diagnosis and to detect signs of complications, measurement of arterial blood pressure, pulse and weight were done.

Laboratory Investigations

A - Routine investigations:

- Kidney function tests (serum creatinine, blood urea, serum uric acid, calcium and phosphorus).
- Liver function tests (serum SGOT, SGPT, ALP, Albumin, total protein, total and direct bilirubin).
- Lipid profile (serum cholesterol, HDL) was performed.
- Heamoglobin %, ESR, CRP were performed.

B - Specific investigation:

- Cadherin 5 & Annexin V Were done by enzyme linked immunosorbant assay (ELISA).

Radiological Investigations

1- ECG (Electrocardiogram).
2- Echo cardiography:
Standard transthoracic M. mode, two dimensional and pulsed wave doppler echocardiograms were obtained soon after a Session of routine haemodialysis using 2.5 MNZ transducer. Leading edge to leading edge measurements were use obtained according to the American Society of Echocardiography recommendation.

Sampling

Blood Samples: Venous blood samples (10 ml) were withdrawn by venupuncture under complete aseptic precautions: 2 ml were transferred to tube containing EDTA anticoagulant for Hb %, 1.6 ml were transferred to tube containing Na citrate tube for ESR and the remaining blood was put in dry tube without use of any anticoagulants. After clotting, serum was immediately separated by centrifugation of the samples at 3,000*g for 10 min and serum was immediately subdivided into 2 eppendorfs one for routine investigations and immediately subjected to assessment of serum creatinine, blood urea, serum uric acid, SGOT, SGPT, ALP, Albumin, total protein, total bilirubin, cholesterol, HDL. And the other for specific investigations (Cadherin 5 & Annexin V) and stored frozen in −20 celsius until the time of assay. Heamolysed, lipaemic samples were discared.

Analytical Methods

All chemical tests were carried out using Dimension auto analyzer (Dade Behring. Inc. S.N.: 97/334W-X. Du pont 1007 Market street, Wilmington, DE 19898, Germany).

Cadherin 5 & Annexin V

There were measured by ELISA.

- Cadherin 5 kit from IBL HAMBURG, Inc. GERMANY. (Flughafenstrasse 52a D-22335, Hamburg, Germany. REF: BE 59111, LOT: 42697019)
- Annexin V kit from HYPHEN BioMed, Inc. FRANCE. (ZAC Neuville Universite—155, rue d'Eragny 95000 Neuville-sur—Oise—France. REF: RK002A, LOT: 080725A)

Statistical Methods

Statistical analysis was performed with the aid of the SPSS computer program (version 14 windows). Results were expressed as means±standard deviation (SD) or number (%). Comparison between the mean values of the three groups (multiple group comparison) was performed using one way analysis of variance (ANOVA). While comparison between each two groups was performed using student's *t* test. Comparison between categorical data [n (%)] was performed using Chi square test. Spearman rank correlation coefficient was used to determine significant correlations among different parameters. Receiver Operating Characteristic (ROC) curve was used to determine the sensitivity and specificity of the assay and the best cut off. *P* value less than 0.05 was considered significant; less than 0.01 was considered

Table 1 Demographic features of different studied groups

	Control (n=20)	CRD without ischemic HD (n=25)[group 1]	CRD with ischemic HD (n=35)[group11]
Age (yrs.)			
Range	41–61	38–64	42–70
Mean±SD	48.85±6.85	53.56±9.94	57.57±7.49*
Gender (F/M)	11/9 (55/45%)	8/17 (32/68%)	11/24 (31.4/68.6%)
Weight (kg)	83.90±9.91	71.72±14.50*	72.06±11.86*
Duration of dialysis (years)	–	5.60±3.51	6.45±4.95

Values are expressed as mean±SD or number (%)

* p<0.01 relative to control group

highly significant and less than 0.001 was considered extremely significant.

Results

Dermographics, clinical and biochemical characteristics of the studied groups are illustrated in Table 1, 2, 3 and 4.

ECG and echocardiography findings of the studied groups are shown in Table 5, 6.

C-reactive protein is 4.85±0.99 in control group which is lower than both group I (26.96±23.40) and group II (63.77±48.24) with statistically significant difference between control group and group1 and II (p value <0.05) and between group 1 and 11 (p value <0.01) [Table 3].

Cadherin 5 is 2.63±1.47 in control group which is lower than both group I (31.69±11.23) with highly statistical significant difference and group II (86.99±21.51) with highly statistical significant difference to both control group and group I (p value <0.01) [Table 7].

Annexin V is 0.47±0.36 in control group which is lower than group I (27.26±11.87) with highly statistical significant difference and group II (83.73±22.64) with highly statistical significant difference to both control group and group I (p value <0.01) [Table 7].

Correlation between Cadherin 5 and Annexin V and some important studied parameters in the studied groups as, EF and CRP were shown in Table 8, 9.

Table 2 Some clinical data of different studied groups

	Control (n=20)	CRD without ischemic HD (n=25)	CRD with ischemic HD (n=35)
SBP (mmHg)	126.00±9.40	136.60±18.18*	137.29±17.29*
DBP (mmHg)	79.25±7.122	86.40±12.543*	84.86±11.146
Pulse	77.70±6.56	84.52±11.38	84.50±18.11
Chest pain	0 (0%)	5 (20%)	22 (62.9%)**,***
L.L.edema	0 (0%)	1 (4%)	17 (48.6%)**,***

Values are expressed as mean±SD or number (%)

* p<0.05; ** p<0.01 relative to control group

*** p<0.01 relative to CRD without ischemic HD group

Table 3 Laboratory data in different studied groups

	Control (n=20)	CRD without ischemic HD (n=25)	CRD with ischemic HD (n=35)
Urea (mg/dl)	33.37±8.69	94.76±32.14*	99.86±43.73**
Creat. (mg/dl)	0.83±0.24	7.41±3. 35**	7.53±3.47**
Uric acid (mg/dl)	4.98±0.83	7.28±1.58**	8.98±3.23**,***
CA (mg/dl)	9.16±0.57	8.96±0.83	8.93±1.10
PO4 (mg/dl)	4.06±0.76	4.86±1.18	6.22±3.27**,***
NA (meq/dl)	139.53±3.55	139.36±3.58	138.07±5.39
K (meq/dl)	4.32±0.48	4.44±0.75	4.63±0.88
RBS (mg/dl)	104.32±23.30	143.08±65.06*	147.60±60.11**
GOT (u/l)	27.58±7.40	19.20±9.26	17.03±6.94
GPT (u/l)	27.21±6.97	28.08±11.35	26.37±9.82
ALP (u/l)	78.58±10.62	192.63±323.04	140.23±84.08
Albumin (g/dl)	4.31±0.56	3.70±0.41	4.64±5.53
Total protien (g/dl)	7.62±0.42	7.06±0.58**	6.96±0.75**
Cholesterol (mg/dl)	146.74±35.68	158.88±35.34	165.71±37.21
HDL (mg/dl)	54.68±6.95	34.00±4.90**	33.89±3.80**
Hb (g/dl)	12.64±1.16	9.83±1.97	12.77±18.61
ESR (1st hr.)	7.53±1.95	72.48±43.34**	79.71±35.04**
ESR (2nd hr.)	14.84±2.34	103.36±39.91**	117.77±31.58**
CRP (%)	1 (5%)	22 (88%)**	35 (100%)**
CRP (mg/dl) (mean±SD)	4.85±0.99	26.96±23.40*	63.77±48.24**,***

Values are expressed as mean±SD or number (%)

* p<0.05; ** p<0.01 relative to control group

*** p<0.01 relative to CRD without ischemic HD group

Table 4 Risk factors in different studied groups

	Control (n=20)	CRD without ischemic HD (n=25)	CRD with ischemic HD (n=35)
History of chronic disease (DM/HTN)	0/0 (0/0%)	7/18 (28/72%)**	12/23 (34.3/65.7%)**
Smoking	1 (5%)	8 (32%)*	15 (42.9%)*

Values are expressed as mean±SD or number (%)

* p<0.05; ** p<0.01 relative to control group

Table 5 ECG & Echocardiography data of different studied groups

	Control (n=20)	CRD without ischemic HD (n=25)[group 1]	CRD with ischemic HD (n=35) [group 2]
ECG			
Normal/IHD/LVH/extrasystoles	20/0/0/0 (100/0/0/0%)	25/0/0/0 (100/0/0/0%)	0/22/1/1 (0/91.7/4.2/4.2%)**,***
EF (%)	–	63.20±8.160	53.91±6.635***
FS (%)	–	35.75±3.756	33.85±6.528
ESD (mm)	–	35.17±8.426	38.82±9.585
EDD (mm)	–	48.11±8.441	49.69±12.166
PWT (mm)		10.58±1.634	12.38±6.168
Dias. Dysf			
SWMA	0 (0%)	0 (0%)	35 (100%)**,***

Values are expressed as mean± SD or number (%)

$^{*}p<0.05$; $^{**}p<0.01$ relative to control group

$^{***}p<0.01$ relative to CRD without ischemic HD group

Comparing Cadherin 5 with Annexin V, there was direct highly significant correlation in both group I and group II (p value <0.01) [Fig. 1].

Comparing Cadherin 5 with ejection fraction, there was indirect highly significant correlation in group I and 11 (r=0.−0.618, p value <0.01).

There was direct highly significant correlation when we compared Cadherin 5 to CRP in both group I and group II [(r=0.716, p=0.01)(r=0.504, p=0.002), respectively].

Comparing Annexin V with ejection fraction, there was indirect highly significant correlation in group I and 11 (r=0.–0.618, p=0.01).

There was direct highly significant correlation when we compared Annexin V to CRP in both group I and group II [(r=0.560, p=0.004) (r=0.462, p=0.005), respectively].

Discussion

Cardiovascular complications are the leading cause of mortality in patients with ESRD. Complications include coronary artery disease, left ventricular hypertrophy, heart failure and arrythmia. Although traditional risk factors, such as diabetes mellitus, hypertension and dyslipidemia are prevalent in ESRD, they are not sufficient to account for the high prevalence of cardiovascular mortality, thus the search for other non traditional risk factors that may be involved in pathogenesis of uremia is under intense study [13].

CVD in CKD is treatable and potentially preventable and so prediction of Cardiovascular complications in CKD patients is very important for prevention and treatment them [14].

Therapeutic interventions in the earlier stages may prevent or ameliorate some of these complications, as well as slow progression to kidney failure [15].

One critical element in the development of atherosclerosis is endothelial dysfunction [16].

Studies have demonstrated that endothelial dysfunction is a predictor of future coronary events and coronary artery disease (CAD) [17].

Assessment of endothelial function may be done by non invasive methods such as measuring plasma levels of Cadherin 5 and Annexin V.

Cadherin 5 is a major determinants of cardiovascular risk in patients with end stage renal failure (ESRF) [18].

Circulating plasma levels of Cadherin 5 in patients at high risk for CHD, as chronic renal patients, were independent predictors of future cardiovascular events [19].

Researchers used the Cadherin 5 assay to quantitate endothelial dysfunction. The method used for measurement of Cadherin 5 is more specific, safe, simple, and rapid. Moreover, the fact that plasma levels of Cadherin 5 independently predicted future cardiovascular events in the present study indicates that measurement of plasma Cadherin 5 levels could be potentially useful for risk assessment of endothelial dysfunction with potential cardiovascular complications [20].

Table 6 Other echocardiographic findings

	Control (n=20)	CRD without ischemic HD (n=25)	CRD with ischemic HD (n=35)
Thickened calcified aortic cusps	0 (0%)	0 (0%)	1 (11.1%)
Conc. Lt ventricular hypertrophy	0 (0%)	0 (0%)	5 (55.6%)
Mitral regurge	0 (0%)	0 (0%)	1 (11.1%)
Pericardial effusion	0 (0%)	0 (0%)	1 (11.1%)
Pulmonary HTN	0 (0%)	0 (0%)	1 (11.1%)

Values are expressed as number (%)

Table 7 Mean cadherin 5 and annexin V levels in different studies groups

	Control (n=20)	CRD without ischemic HD (n=25)	CRD with ischemic HD (n=35)
Cadherin 5 (ng/ml)	2.63±1.47	31.69±11.23*	86.99±21.51*,**
Annexin V (ng/ml)	0.47±0.36	27.26±11.87*	83.73±22.64*,**

Values are expressed as mean±SD

* p<0.01 relative to control group

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 [21].

In the present study, the major aim was to evaluate the role of Cadherin 5 and Annexin V as sensitive, specific, prognostic and predictive markers for cardiovascular complications in CKD patients.

We found a significant increase in CRP in group II (63.77±48.24) versus the control group (4.85±0.99) and group I (26.96±23.40) with but highly statistical significant difference (p value <.01). significant increase in CRP in the hemodialysis group versus the renal impairment group and versus the control group (P<0.001). In agreement with our results Jimenez et al. [22] reported that there is an evidence that patients with CKD are in a state of chronic inflammation with activation of C-reactive protein and proinflammatory cytokines and is associated with increased oxidative stress and endothelial dysfunction.

In our study, Cadherin 5 was 2.63±1.47 in control group which is lower than both group I (31.69±11.23) with highly statistical significant difference and group II (86.99±21.51) with highly statistical significant difference to both control group and group I.

Table 8 Correlation between Cadherin 5 and some important studied parameters in the studied groups

Variable correlated	CRD without ischemic HD		CRD with ischemic HD	
	R	p value	R	p value
Annexin V	0.842	0.01**	0.884	0.01**
EF	−0.618	0.01**	−0.106	0.550 NS
FS	0.131	0.543 NS	−0.041	0.819 NS
CRP	0.716	0.01**	0.504	0.002**
ESR 1st hr	0.320	0.119 NS	0.386	0.022*
ESR 2nd hr	0.283	0.170 NS	0.406	0.016*

r=correlation coefficient

NS (p>0.05)=not significant; *p<0.05=significant; **p<0.01=highly significant

Table 9 Correlation between Annexin V and some important studied parameters in the studied groups

Variable correlated	CRD without ischemic HD		CRD with ischemic HD	
	R	p value	R	p value
EF	−0.556	0.004**	−0.117	0.509 NS
FS	0.043	0.842 NS	−0.097	0.586 NS
CRP	0.560	0.004**	0.462	0.005**
ESR 1st hr	0.253	0.223 NS	0.256	0.137 NS
ESR 2nd hr	0.225	0.280 NS	0.296	0.084 NS

r=correlation coefficient

NS (p>0.05)=not significant; **p<0.01=highly significant

These results correlate with Bulut et al. [23] who discovered that apparently healthy young men with a family history of premature coronary artery disease show elevated levels of circulating endothelial microparticles, when compared to young men without a positive family history. This finding appears to precede the development of endothelial dysfunction.

These results also in correlate with Bernard et al. [24] who found an association between plasma EMP-CD144+ (Cadherin 5) and coronary noncalcified atheroma in a population of type 2 diabetic patients. EMP were characterized using anti-CD144 (VE-cadherin) antibody since it is the most specific marker for endothelial cells, and since it was established that CD144-positive EMP derived selectively from human endothelial cells. They found an increase in MP in diabetic patients compared with nondiabetic controls. They reported a significant elevation of EMP CD144+ in type 2 diabetic patients with ACS compared with type 2 diabetic patients without metabolic syndrome. They found a

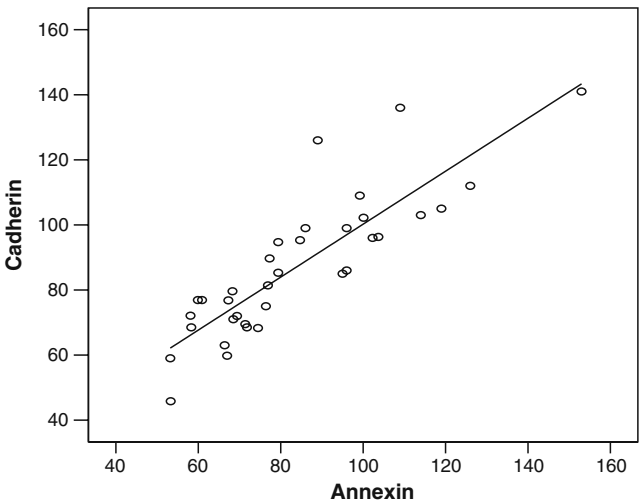

Fig. 1 Correlation between cadherin and annexin V in CRD with ischemic HD (r=0.884, p=0.01**)

significant association between EMP and noncalcified coronary diseased segments.

Also Soeki et al. [8] demonstrated that the soluble VE-cadherin (Cadherin 5) concentration correlates with the extent of coronary atherosclerosis independently of other atherosclerotic risk factors including sex, age, hypertension, diabetes mellitus, smoking and the lipid profiles. Among the 'traditional'atherosclerotic risk factors, diabetes and high-density lipoprotein-cholesterol independently correlated with the extent of coronary atherosclerosis, but these factors were secondary to VE-cadherin in predicting the severity of coronary atherosclerosis. These results suggest that the plasma VE-cadherin concentration might be one of the most useful indicators of cardiovascular disease, such as atherosclerosis, independent of other risk factors.

In our study, Annexin V was 0.47±0.36 in control group which is lower than group I (27.26±11.87) with highly statistical significant difference and group II (83.73±22.64) with highly statistical significant difference to both control group and group I (p value <0.01).

These results correlate with Faure et al. [25] who showed that the levels of annexin V+ MP, estimating the number of MP whatever their cellular origin, were significantly increased in both CRF and HD patients.

These results also were in correlate with Ravassa et al. [26] who said that Annexin V is upregulated in hypertensive patients with LVH either without or with HF.

However, these results is not correlating with Tits et al. [27] who hypothesized that the plasma level of endogenous annexin A5 is inversely associated with the presence and extent of atherosclerotic plaque formation due to binding of annexin A5 to components of the atherosclerotic plaque. Meanwhile, Aikawa and Libby [28] and Cederholm et al. [29] said that Vulnerable plaque is characterized by an increased degree of inflammation and a high propensity to overimposed thrombosis. A rupture within a lesion can initiate thrombus formation, but also possibly more subtle events, such as surface microerosions caused by detachment of apoptotic endothelial cells, shedding microparticles or by microthrombosis within the neovasculature of plaque shoulder region, can lead to acute obstruction and clinical manifestations. We have discovered abundant presence of annexinA5 in advanced atheromas from general population.

Comparing Cadherin 5 with Annexin V, there was direct highly significant correlation in both group I and group II (p value <0.01). and that agree with Jy et al. [30] who stated that Cadherin 5 and Annexin V are largely involved in atherogenesis and can be used for quantitative risk assessment of atherosclerosis.

An inverse correlation was found between Cadherin 5 and Annexin V and ejection fraction in group I (p value <0.01). This is in agreement with the study done by Zoccali et al. [31] who reported that high Cadherin 5 and Annexin V were associated with systolic dysfunction and left ventricular failure and such increase is proportional to the severity of the disease.

There was direct highly significant correlation when we compared Cadherin 5 and Annexin V to CRP in both group I and group II (p value <0.01). However these results is not correlating with Boulanger et al. [32] who proved that there is weak correlation between EMP & CRP. Meanwhile Nakajimi et al. [33] proved that CRP is associated with endothelial cell dysfunction and progression of atherosclerosis, possibly by decreasing nitric oxide synthesis. Also it has the ability to sensitize endothelial cells to destruction by cytotoxic CD4 T cells. In addition CRP facilitates thrombogenesis through stimulation of tissue factor biosynthesis by macrophages.

We found a significant decrease in HDL-cholesterol in group I (34.00±4.90) and group II (33.89±3.80) in comparison with control group (54.68±6.95) with highly statistical significant difference (p value <0.01). In harmony with our study Krane and Wanner [34] reported that chronic kidney disease is associated with a highly atherogenic lipid profile, characterized by elevated triglycerides, low HDL cholesterol.

Regarding our echocardiographic findings we found:

- A significant decrease in Ejection fraction in group I (63.20±8.160) versus group II (53.91±6.635) (P<0.01).
- 100% of group II had wall motion abnormality.

  These agree with studies that considered CKD patients in all stages of CKD are considered in the highest risk group for development of CVD [35], and also agree with Ix et al. [36] whom reported that chronic renal impairment (CRI) was associated with coronary artery disease which was evaluated by exercise stress echocardiography which was used to identify inducible ischemia, defined as any wall motion abnormality seen at stress but not at rest. They found that CKD is strongly associated with exercise-induced ischemia in patients with coronary artery disease.
- 36% of group I and %74.2 of group II had diastolic dysfunction In harmony with our results Nardi et al. [37] stated that patients with chronic renal insufficiency have a much greater cardiovascular risk than the general population. Moreover, hypertension is common in these patients, so as left ventricular hypertrophy and diastolic dysfunction, which contribute to a worse prognosis. Data showed a greater alteration of diastolic function in group II, in part independent of LVH.
- 31.4% of group II had left ventricular hypertrophy. In agreement with our results Kimura et al. [38] stated that LVH, which is a strong predictor of mortality in patients with end stage renal disease, is present in over

70% of patients commencing dialysis. Increased left ventricular mass is associated with severe renal dysfunction and a higher cardiovascular mortality [35].

Conclusion

Endothelial dysfunction leading to atherosclerotic vascular lesions in patients with CKD can be assessed quantitatively by measurement of plasma levels of Cadherin 5 and Annexin V.

Recommendation

A longitudinal study is recommended with CRD without ischemic HD by watching the levels for Cadherin 5 and Annexin V. It would of interesting to see the levels of these two markers just before the ischemia in CRD patients. Then one can use these two as markers to predict the outcome of cardiac complications and possibly prevent it in CRD patients.

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