

Sex Disparity in Cardiovascular Mortality in Patient with End-Stage Renal Disease and Type 2 Diabetes Mellitus

Essam Nakhla¹, Ruchi Singh², Sudha Chennasamudram², Olga Vasylyeva¹, Tarek Naguib¹ and Tetyana L Vasylyeva^{2*}

¹Department of Internal Medicine Texas Tech University Health Sciences Center, Amarillo, Texas, USA

²Department of Pediatrics, Texas Tech University Health Sciences Center, Amarillo, Texas, USA

Abstract

Aim: We hypothesized that sex differences relative to the pro-inflammatory and endothelial markers may exist and play a role in the higher CVD mortality of women with End-Stage Renal Disease and Type 2 Diabetes Mellitus.

Methods: 15 subjects (average age of 54 ± 9 years, 8 females and 7 males) on Peritoneal Dialysis (PD) were enrolled. Evaluation of Endothelial Function (EF) was done by Reactive Hyperemia Index (RHI). Serum levels of EF biomarkers: Endo Thelin (ET) 1, Plasminogen Activator Inhibitor 1 (PAI-1), soluble Vascular Adhesion Molecule 1 (sVCAM) and soluble Inter Cellular Adhesion Molecule 1 (sICAM); and inflammatory markers: Inter Leukin 6 (IL-6), IL-1 β , Tumor Necrosis Factor alpha (TNF- α) and C- Reactive Protein (CRP) were measured. A non-parametric Wilcoxon test was performed for all the study parameters.

Conclusion: The combined increase of IL-1 β and ET1 found in the females of this study population reflects significant sex difference in the expression of these inflammatory markers that may actually be responsible for the increase in CVD mortality among diabetic females with ESRD on PD. Larger scale studies are warranted to confirm this conclusion that may guide future studies to control CVD mortality.

Keywords: Gender Difference; Cardiovascular Disease; Inflammatory Markers; ESRD; Type 2 Diabetes Mellitus

***Corresponding Author:** Tetyana L Vasylyeva, Texas Tech University Health Sciences Center, School of Medicine, Department of Pediatrics, 1400 Coulter Street, Amarillo, Texas, USA; E-mail: tetyana.vasylyeva@ttuhsc.edu

Background

Patients with Diabetes Mellitus (DM) have greater chances of Cardio Vascular Disease (CVD) than the general population [1]. Diabetic women displayed a higher risk for Myocardial Infarction (MI) than diabetic men [2,3]. When women developed a MI with ST-segment-elevation, younger women were found to have higher mortality compared with men, despite the lack of significant difference in the management of the disease between the sexes [4]. Of interest, young women with coronary heart disease actually have more ischemia and mortality than men, despite having less obstructive disease. This suggests that cardiac ischemia in women is likely more related to microvascular dysfunction, probably due to coronary reactivity and endothelial dysfunction [5].

A study of the National Registry of Myocardial Infarction revealed that among hospitalized patients, women were found to be more likely to present without chest pain but had higher mortality than men of the same age group. There was

no obvious reason for this discrepancy that has become more pronounced in younger age patients [6,7]. Also, the US Renal Data Systems Annual report reveals that women with End-Stage Renal Disease (ESRD) who are younger than 70 years of age suffer more mortality from MI than men in the same age group and that sudden cardiac death among persons with Diabetes Mellitus Type 2 (T2DM) and ESRD was highest compared to all other primary ESRD diagnoses [8-10].

The sex differences in CVD pattern and mortality do not have a readily acceptable explanation. However, it is worth noting that higher levels of interleukin-1 beta (IL-1 β) and Endo Thelin-1 (ET-1) have been reported in CVD [2, 4]. Therefore, we hypothesized that sex differences relative to the pro-inflammatory and endothelial markers may exist and play a role in the higher CVD mortality of women in this high risk population.

Material and Method

Study Design and Patient Population

This is the post-hoc analysis of our previous an open-label, randomized crossover study that was designed to evaluate the effect of sevelamer versus calcium carbonate binders on the inflammatory markers. However, only baseline blood levels of inflammatory markers were used for the purpose of this study. Fifteen subjects with T2DM on peritoneal dialysis whose serum phosphate levels were > 5.5mmol/L were enrolled from Amarillo Kidney Specialist, LLC dialysis unit. The study protocol was approved by Texas Tech University, Health Sciences Center, Institutional Review Board and all the patients signed informed consent forms, consistent with the Declaration of Helsinki.

Subjects with Para Thyroid Hormone (PTH) levels greater than 1,000 mmol/L, calciphylaxis, and history of hypercalcemia were excluded from the study. Subjects then had Endothelial Function (EF) evaluated as reactive Regional Hyperemia Index (RHI) and serum levels for ET-1, Plasminogen Activator Inhibitor-1 (PAI-1), soluble Vascular Adhesion Molecule (sVCAM), soluble Inter Cellular Adhesion Molecule (sICAM). Pro-inflammatory cytokines levels in serum were measured for

InterLeukin-6 (IL-6), IL-1 β , Tumor Necrosis Factor alpha (TNF- α), and C-Reactive Protein (CRP).

Measurement of serum sVCAM, sICAM, ET-1 and PAI-1

Enzyme-Linked Immuno Sorbent Assays (ELISA) was used according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN) to measure the serum levels of sVCAM, sICAM, ET-1 and PAI-1.

Measurement of serum pro-inflammatory cytokines

IL-6, IL-1 β , TNF- α , and CRP were measured by ELISA according to the manufacturer's instructions (R&D Systems, Inc. Minneapolis, MN).

Reactive Hyperemia Index

Vessel stiffness, which reflects EF, was measured by Reactive Hyperemia Index (RHI) using peripheral arterial tonometry by Endo-PAT 2000, as previously described [11]. Normal RHI is more than 1.67.

Life time cardiovascular risk factor was calculated by "QRISK®-lifetime cardiovascular risk calculator".

Statistical analysis

ELISA data was analyzed by using 4 parameter logistic equations. Subsequently a non-parametric Wilcoxon test was used to compare all the study parameters. The results are presented as means $\pm$ standard deviation of the means. Spearman correlation was calculated between ET-1 and other inflammatory markers in both groups. Statistical significance was set at $P < 0.05$. Data was analyzed using IBM SPSS version 20.0 (SPSS, Inc., Chicago, IL).

Results

Sex difference and Markers of Endothelial Dysfunction

The main demographic and clinical characteristics of the subjects are listed in Table 1. Life time cardiovascular risk factor was significantly high in male as compared to female. Serum concentration (pg/mL) of endothelial function markers (sVCAM, sICAM, ET-1 and PAI-1) are listed in Table 2. A

serum level of ET-1 was found significantly high in females as compared to males. However, there were no statistically

significant differences found in the other tested markers viz. sVCAM, sICAM and PAI-1 with respect to the sex difference.

Table 1: Baseline parameters; *p (two tailed) ≤ 0.05 .

	Male	Female	p value
Age (Years)	48.5 $\pm$ 11.17	58.3 $\pm$ 9.47	0.06
BMI	32.78 $\pm$ 5.9	35.1 $\pm$ 5.45	0.33
Chlosterol (mg/dL)	153 $\pm$ 27.02	173.9 $\pm$ 42.84	0.07
Triglyceride (mg/dL)	189 $\pm$ 128.63	233.4 $\pm$ 104.03	0.30
HDL (mg/dL)	38 $\pm$ 9.80	36.4 $\pm$ 11.04	0.40
LDL (mg/dL)	74.87 $\pm$ 34.03	84.2 $\pm$ 40.97	0.18
Chlosterol / HDL ratio	4.78 $\pm$ 2.07	5.6 $\pm$ 61.69	0.19
HbA1C (mmol/mol)	6.61 $\pm$ 3.26	7.6 $\pm$ 2.94	0.16
Lifetime cardiovascular risk %	56.30 $\pm$ 9.86	44.11 $\pm$ 5.40	0.01*

Table 2

Markers	Males	Females	p value
PAI-1	2.47 $\pm$ 0.28	2.99 $\pm$ 0.37	0.157
ET-1	1.98 $\pm$ 0.21	2.38 $\pm$ 0.44	0.035*
sICAM	51.14 $\pm$ 8.2	35.87 $\pm$ 4.9	0.327
sVCAM	53.37 $\pm$ 18.22	101.06 $\pm$ 27.26	0.190

Table 2: Markers of endothelial function (M $\pm$ SEM); PAI-1: plasminogen activator inhibitor-1; ET-1: endotheline-1; sICAM: soluble intercellular adhesion molecule; sVCAM: soluble vascular adhesion molecule.

Serum concentration of all markers were measured in ng/mL

*p (two tailed) ≤ 0.05 .

Sex difference and Levels of Serum pro-inflammatory cytokines

Serum concentration of IL-1 β was significantly higher in females (p = 0. 03) as compared to males, meanwhile TNF α was slightly higher in males (p = 0.05) (Figure 1A and C). There

were no statistically significant differences found in IL-6 and CRP with respect to the sex difference (Figure 1B and D).

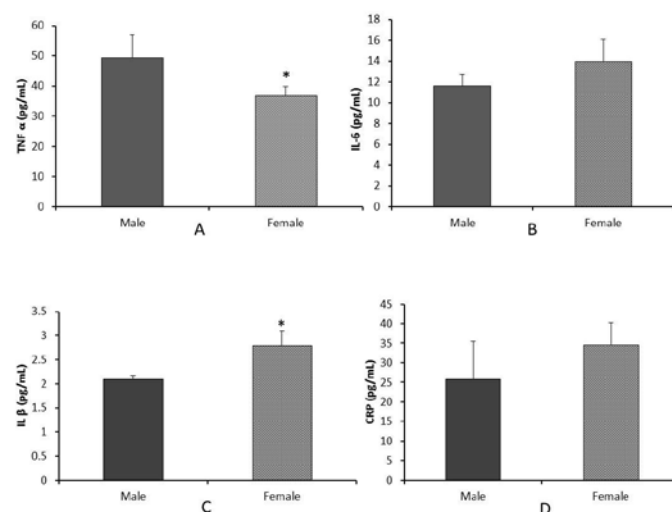


Figure1: Pro-inflammatory markers in males vs. females. **A:** Comparison of serum concentration of TNF α in both groups; **B:** Comparison of serum concentration of IL-6 in both groups; **C:** Comparison of serum concentration of IL β in both groups; **D:** Comparison of serum concentration of CRP in both groups.

Reactive Hyperemia Index (RHI) and Sex difference

Although average RHI was not significantly different between male vs. female groups (1.79 ± 0.19 vs. 1.55 ± 0.22 ; $p = 0.40$), male average RHI was > 1.67 , which is considered normal and female RHI was under the abnormal range.

Correlation of ET-1 and other inflammatory markers and Sex difference

ET-1 was found to be strongly positive and significantly correlated with sVCAM and IL-6 in females. However, no such correlations were observed in males (Table 3).

Females							
	PAI-1	sICAM	sVCAM	TNF α	IL-6	IL β	CRP
ET-1	-0.06	-0.23	0.83	-0.34	0.766	0.33	0.276
p value	0.9	0.62	0.02*	0.44	0.04*	0.46	0.54
Males							
	PAI-1	sICAM	sVCAM	TNF α	IL-6	IL β	CRP
ET-1	0.575	-0.429	0.409	-0.09	-0.13	-0.73	-0.112
p value	0.18	0.34	0.36	0.84	0.78	0.06	0.81

Table 3: Correlation of Endo Theline – 1 (ET-1) and other inflammatory markers and sex difference. PAI-1: plasminogen activator inhibitor-1; sICAM: soluble intercellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; TNF α : tumor necrosis factor alpha; IL-6: interleukin-6; IL β : interleukin β ; CRP: C-reactive protein

*p (two tailed) ≤ 0.05 .

Serum concentrations of PAI-1, sICAM, sVCAM and CRP were measured in ng/mL. Serum concentrations of TNF α , IL-6, IL β and ET-1 were measured in pg/mL.

Correlation of peripheral leukocyte populations and other inflammatory markers and sex difference

Pearson correlation between peripheral leukocyte populations (lymphocytes and neutrophils) and inflammatory markers was performed (Table 4). There was no significant correlation observed in conjunction with lymphocytes in both male and female. However, in females, neutrophils were significant and strongly correlated with sICAM, TNF α and CRP.

	ET1	sICAM	IL-6	PAI-1	TNF α	sVCAM	IL β	CRP
Male								
Lymphocytes	-0.02	0.53	-0.04	-0.02	0.57	-0.71	-0.07	0.43
Neutrophils	0.03	-0.63	0.11	-0.08	-0.29	0.52	0.31	-0.32
Female								
Lymphocytes	0.41	-0.68	-0.07	-0.43	-0.32	0.69	0.14	0.26
Neutrophils	-0.49	0.90*	-0.53	0.75	0.87*	-0.61	0.67	0.80*

Table 4: Correlation of peripheral leucocytes population (lymphocytes, neutrophils) and sex difference. PAI-1: plasminogen activator inhibitor-1; sICAM: soluble inter cellular adhesion molecule; sVCAM: soluble vascular adhesion molecule; TNF α : tumor necrosis factor alpha; IL-6: interleukin-6; IL β : interleukin β ; CRP: C-reactive protein

*p (two tailed) ≤ 0.05 .

Serum concentrations of PAI-1, sICAM, sVCAM and CRP were measured in ng/mL. Serum concentrations of TNF α , IL-6, IL β and ET-1 were measured in pg/mL.

Discussion

We analyzed data obtained from patients in one of our community dialysis centers to shed light on the underlying inflammatory process in CVD and diabetic patients on PD. The goal was to identify potential factors that may explain the sex difference in CVD mortality in this high risk population.

We hypothesized that sex difference in pro-inflammatory and endothelial function markers exist and plays a significant role in CVD mortality among women who were 65 years old or younger and who are receiving PD for managing ESRD. The study population seemed to be appropriate in light of the previous work outlined above and the work implicating inflammatory markers in CVD mortality in peritoneal dialysis [12,13]. Lifetime CVD risk calculator showed that there is more risk in male than female. However, CVD behaves differently in females than males with a higher burden of mortality particularly in the context of ESRD and diabetes [14]. Therefore there is a need to improve conventional methods to calculate cardiovascular risk factors, which are also renal function based.

The results of the study revealed statistically significant higher levels of IL-1 β and ET-1 in females as compared with males. There were no statistically significant differences found in the other tested markers, however. These findings merit a closer examination of the literature for potential plausibility.

IL-1 β was isolated in high levels from endothelial cells of arterial wall of atherosclerotic arteries in ischemic cardiomyopathy when compared to non-ischemic cardiomyopathy patients. Levels were also found to be directly proportional to the severity of atherosclerosis and consequently to disease severity and progression [15]. Beside the strong correlation between IL-1 β and atherosclerosis, IL-1 β has also a direct depressing effect on the cardiac muscle cell. The effect is thought to be mediated by ceramidase or nitrous oxide synthase enzyme inhibition. This is both a direct effect of IL-1 β and also a synergistic effect with TNF [16]. Of interest, the protease caspase-1, which is responsible for the processing of IL-1 β into an active molecule, is proposed as a cell death enzyme and executes a rapid program of cell death, termed pyroptosis, in macrophages[17].

Not only does IL-1 β worsen the ischemia related injury in advanced coronary atherosclerosis, and is secreted by cardiac muscles during ischemia, but also alteration in gene expression in response to IL-1 β is described to resemble, in many ways, the phenotype of the failing heart [18]. Also, IL-1 β receptor antagonist, introduced by gene transfection into a rat heart, protected the myocardium from ischemia reperfusion injury by attenuating the inflammatory response and decreasing the infarct size [19].

In T2DM, elevated IL-1 β was found to strongly promote B cell inflammation, dysfunction and death. In vivo, the balance between the levels of IL-1 β , with its pro inflammatory effect and the naturally occurring IL-1 β receptor antagonist, is thought to determine the outcome of B cell inflammation and death, which is seen clinically as the degree and progression of T2DM [20]. There is also rising evidence that administration of the IL-1 β receptor antagonist, anakinra, as solo or add-on therapy improves the glycemic control in T2DM as it decreases the markers for systemic inflammation [21].

A statistically significant correlation between ET-1 levels and aortic calcium scores were established in a cohort retrospective study. ET-1 was directly involved and related to calcified plaque formation as an independent factor. The level of ET-1 in serum determined the lesion severity. The study

concluded that ET-1 can even be used as an independent risk stratifying factor for Coronary Artery Disease (CAD) in asymptomatic patients who had angiographic evidence of atherosclerosis as detected by multi detector computed tomography. It also can be used as an independent predictor of appearance of symptoms in those patients at risk of CAD [22]. In our study, ET-1 was found to significantly correlate with IL-6 and sVCAM, only in female patients.

A great deal of clinical data supports the theory of an essential role of ET-1 in the development of atherosclerosis and CAD in more than one mechanism. One mechanism is the promotion of endothelial dysfunction and arterial wall injury; another is the induction of vasoconstriction of the coronary arteries and their vasa vasorum; an effect mediated by both types of ET-1 receptors, types A and B. Though, receptor A mediates vasoconstriction and hypertension, receptor B, which is more expressed in females, actually is protective against hypertension [23]. Casimir et al. in 2010 have demonstrated that there is a relationship between sex and both the production of inflammatory markers and neutrophil recruitment [24]. We have gotten similar results. Slightly higher levels of TNF- α were also found in males. TNF- α is a cytokine that is produced primarily by monocytes and macrophages, with a key role in inflammatory responses, in addition to the effects in other tissues, including adipocytes [25]. TNF- α may relate to BMI and increased muscle mass [26].

Accordingly, both IL-1 β and ET-1 in concert with other inflammatory molecules appear to play a significant role in arterial wall injury and atherosclerosis development, and may consequently increase mortality in the context of ischemic heart disease.

The combined increase of IL-1 β and ET-1, found in the females in our study population, reflects significant sex difference in the expression of these inflammatory markers that may actually be responsible for the increase in CVD mortality among diabetic females with ESRD on PD. Large scale studies are warranted to confirm this conclusion and guide future studies to control CVD mortality.

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