

# Homocysteine and Malondialdehyde (MDA) Levels Associated with the Occurrence of Cardiovascular Disease (CVD) in Chronic Renal Failure (CRF) in Malaysia

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## Abstract

**Introduction:** Atherosclerosis and following cardiovascular disease (CVD) are known as important reasons of the increased morbidity and mortality observed in patients with chronic renal failure (CRF). The association of serum malondialdehyde (MDA), homocysteine as well as other cardiovascular risk factors in relation to existence and cause of CVD were investigated.

**Methods:** In these study 66 CRF patients without dialysis and 107 patients receiving dialysis were recruited and further stratified into group with CVD and without CVD as case groups. Those without renal failure and CVD were assigned as control group (n=33).

**Results:** The patients with CRF showed a significant increase in plasma levels of MDA, homocysteine and C-reactive protein (CRP) compared to control. The positive association were observed between homocysteine, creatinine and MDA (all  $p < 0.01$ ) and another positive association were between CRP and age, creatinine and MDA (all  $p < 0.05$ ). Analysis of association risk factors showed that only age, CRP and lipid profile had significant association with CVD events.

**Conclusion:** The results demonstrated elevation in plasma values of MDA, homocysteine and CRP in patients with CRF, with or without CVD. However, these modifications may be lead to atherosclerosis and consequence CVD event. These parameters may be important with respect to the high morbidity and mortality of CVD found in patients with CRF.

**Keywords:** Chronic Renal Failure (CRF), CVD, Hyperhomocysteinemia, Oxidative Stress

## 1. Introduction

Atherosclerosis and CVD play an important role as a major source of morbidity and mortality in CRF patients. In addition, recent studies suggest that, cardiovascular injury started during early development of CRF complications (Nagane, 2009). Although traditional Framingham risk factors such as hypertension, dyslipidemia and diabetes may account for a large part of the excessive burden of CVD in CRF patients, non-traditional risk factors such as inflammation, oxidative stress and hyperhomocysteinemia may also contribute to the outcome of CVD in CRF patients (Stenvinkel *et al.*, 2003).

Several epidemiological studies have reported that elevated total plasma homocysteine is a risk factor for cardiovascular disease. Homocysteine contains a reactive sulfhydryl group, and undergoes oxidation to disulfide at physiological pH in the presence of O<sub>2</sub> (Mallamaci *et al.*, 2002). It has been suggested that this pro-oxidant activity of homocysteine is also responsible for the oxidation of LDL cholesterol and damaging of vascular cells and tissues (Landry *et al.*, 2006).

Hyperhomocysteinemia and lipid abnormalities are commonly found in patients with CRF; both are recognized as risk factors for atherosclerosis (Dom *et al.*, 2003). Inflammation plays a central role in the pathogenesis of atherosclerosis in CRF patients. C-reactive protein (CRP) level was found to be elevated in kidney failure and may be related to cardiovascular complications (Menon *et al.*, 2003; Wong *et al.*, 2007). Due to the high mortality and morbidity rate in CRF patients caused by CVD, it is vital to find way which could reduce the death toll in these patients.

The current study was conducted to determine the role of non-traditional risk factors in the development of cardiovascular disease in dialysis and pre-dialysis patients. In this regard, biomarker of oxidative stress, homocysteine, CRP and lipid profile were measured.

## 2. Material and Method

### 2.1 Subjects

Individuals with CRF, with and without CVD from Serdang Hospital Selangor, Malaysia were recruited for this study. Patients with CRF were selected base on glomerular filtration rate (eGFR), CKD stage 1-4 (pre-dialysis) and CKD stage 5 (dialysis) were recruited (Levey *et al.*, 2002). The selected population consisted of 85(49%) male 88 (51%) females, age between 18–80 years. Out of 173 patients, 66 were CRF patients with GFR (glomerular filtration rate) more than 15ml/min/1.73m<sup>2</sup> pre-dialysis whereas 107 patients with GFR less than 15 ml/min/1.73m<sup>2</sup> receiving dialysis and were clinically stable and free of active infections or autoimmune diseases. Those receiving immunosuppressive treatment and non-steroidal anti-inflammatory drugs were excluded from the study. Seventy six CRF patients had clinical symptoms or a previous history of cardiovascular disease (history of coronary heart disease or myocardial infarction, chronic ischemic heart disease, atherosclerosis, ischemic heart disease and congestive heart failure). The control group consist of 33 healthy subjects between the age of 18-80 years, who had no history of CRF or CVD. The study was approved by the Ethic Committee of Faculty of Medicine and Health Science, University Putra Malaysia (UPM). The informed consent was obtained from the subjects before participating in this study.

### 2.2 Blood sampling and laboratory measurements

Blood samples from CRF patients and controls were taken under fasting condition into tubes containing EDTA. The plasma were prepared conventionally, aliquoted and stored at -80° C until the analysis were carried out.

### 2.3 Routine parameters

Lipid profile (HDL-C, LDL-C, total Cholesterol, triglyceride) were determined by enzymatic method using Roche/Hitachi analyzer (Roche Diagnostic, USA).

C-reactive protein (CRP) was measured by Selectra Flexor/XL autoanalyzer. This assay was based on the principle of particle-enhanced immunological agglutination or immunoturbidimetric assay. Following agglutination, this was measured turbidimetrically. Assay principle homocysteine was an enzyme immunoassay for the determination of total homocysteine in blood with an ELISA kit (AXIS, EIA package Insert, UK). MDA was measured using the established thiobarbituric acid (TBARS) method. MDA level of the plasma was measured according to the Tukožkan *et al.* (2006). The standards of 1,1,3,3 tetraetoxypropane (TEP) were used for preparation of standard curve. The results were expressed as nmol/ml plasma.

### 2.4 Statistical analysis

The results were presented as the means ± SD for normal and median (range) for skewed distributed variables. The significant differences of normal distributed variables between 2 groups were assessed by t-test and analysis

of variance (ANOVA), while variables with non-Gaussian distribution were compared by using nonparametric Mann–Whitney and Kruskal–Wallis tests, respectively. All  $p$ -values were two-tailed and differences were considered significant if  $p$ -values were  $\leq 0.05$ . The association between the dependent variable and independent variables was determined by the correlation test. Associations of risk factors for CVD outcome by using logistic regression analysis was performed between two groups of CRF, with and without CVD as the dependent variable. All anthropometric, physiological and biochemical variables were incorporated and finally association of several risk factors with CVD outcome in CRF patients were evaluated.

### 3. Results

#### 3.1 Descriptive and ANOVA statistic

The prevalence of demographic and clinical characteristics observed variables in CRF patients and healthy control are summarized in Table 1. There were significant differences in mean of the age in CRF and dialysis with CVD compared with control group. Dialysis groups had higher duration of CRF compared to group pre-dialysis. All groups showed no significant difference in systolic blood pressure but group with CRF without CVD had significantly high level of diastolic blood pressure compared to other groups. There were significant different in the antihyperlipidaemic and hypoglycaemic drugs consumption. CRF group without CVD had significant highest consumption of antihyperlipidemic drug, as well as CRF group with CVD for hypoglycaemic drug compared to other groups. As shown in Table 2, MDA concentrations were significantly different between CRF and control group. Among those involved in the study, CRF group receiving dialysis with CVD had the highest mean of MDA value. The levels of serum C - reactive protein in different groups of CRF was significantly higher than control, with CRF receiving dialysis and having CVD has the highest compared to other groups. Homocysteine concentrations in different group of CRF were significantly higher than control and CRF group receiving dialysis had higher levels of homocysteine compared to other groups. Plasma total cholesterol was significantly higher in all CRF groups compared to control group. Plasma triglyceride was only different in CRF without CVD compared to control group. However, there were no significant changes in level of HDL and LDL in all CRF patients compared to control group.

#### 3.2 The Pearson correlation coefficients ( $r$ ) analysis

The Pearson correlation coefficients ( $r$ ) of homocysteine, CRP and MDA levels relative to other parameters were carried out. Plasma level of homocysteine was positively and significantly associated with creatinine and MDA levels with ( $r=0.279$ , and  $r=0.276$ , both  $p<0.01$ ) respectively. The positive and significant correlation was observed between CRP and age, creatinine, MDA and homocysteine levels with ( $r=0.174$ ,  $r=0.130$ ,  $r=0.157$  and  $r=0.155$ , all  $p<0.05$ ) respectively and inversely, correlated with HDL. The correlation of MDA concentration was positively and significantly with age ( $r = 0.248$ ,  $p < 0.01$ ), homocysteine ( $r = 0.276$ ,  $p < 0.01$ ), and negatively with HDL ( $r = - 0.162$ ,  $p < 0.05$ ).

#### 3.3 Associations of Risk Factors for CVD outcome by using Logistic regression analysis

Logistic regression analysis was performed between two groups' CRF without CVD and CRF with CVD as the dependent variables (Table 4). All anthropometric and biochemical variables were keyed in; however, only age (OR=1.003, CI 1.000-1.005,  $p<0.001$ ), CRP (OR= 1.010, CI 1.000-1.003,  $p<0.05$ ) and lipid profile showed significant association between these variables and occurrence of CVD. These associations in age, CRP, cholesterol, LDL and triglyceride showed strong regression in adjusted odds ratio.

#### 3.4 Evaluation of longitudinal study end point of cardiovascular disease

In longitudinal study, type of cardiovascular event (MI, death, stroke and revascularization) were examined among the CRF patients with complete follow-up, which data are available. At the end of one year observation, of 173 CRF patients, 14 patients (8%) were died. Of these, seven patients of CRF with CVD receiving haemodialysis which account 4% of the sample number died. On the other hand, 2 CRF without CVD patients (1.2%) receiving CAPD (continuous ambulatory peritoneal dialysis), 1 patient of pre-dialysis without CVD (0.6%) and 4 patients in pre-dialysis with CVD (2.4%) died within that one year period (Table 5). In one whole year follow up only 1 patient (0.6%) belong to the haemodialysis and another one of pre-dialysis with CVD (0.6%) had myocardial infarction (MI). Only one of haemodialysis with CVD patient had revascularization (0.6%).

### 4. Discussion

The results of the present study indicated that CRF patients had significantly increased levels of homocysteine, CRP and MDA as non-traditional risk factors compared to control subjects which are in agreement with other reports (Nagane, 2009; Vaziri, 2004).

#### 4.1 Socio-demographic and clinical characteristic of CRF patients

The results in this study revealed that duration of CRF complication were much longer especially in CRF patient with dialysis and CVD than other groups. Similarly, patient in our hospital were much older in dialysis group with CVD. Logistic regression analysis also showed, there were significant relationship between age and prevalence of CVD in CRF patients. These results support the observations in other study of Brenda and co-workers (Brenda *et al.*, 2007) that, renal insufficiency is a marker for elevated cardiovascular disease risk in community dwelling with elderly adults. The most interesting observation in the present study was the presence of direct correlation between CVD prevalence and aging-associated inflammation. This result agrees with Pawlak and co-worker studies (Pawlak *et al.*, 2005).

#### 4.2 Biochemical parameters of CRF patients

There are several observational studies examining the relationship between dyslipidemia and CVD outcome (Shoji *et al.*, 2001). Logistic regression analysis showed that CRF patients with high levels of triglyceride, LDL and cholesterol had significant association with the occurrence of CVD. The results of present study agreed with other investigators revealed those classic risk factors of atherosclerosis, age, duration of CRF and elevated lipid profiles, contribute to the development of carotid atherosclerosis in patients with CRF (Shoji *et al.*, 2001; Leskinen *et al.*, 2004). Determination of MDA, as the product of the oxidation of polyunsaturated fatty acid was reported to be an appropriate indirect method that revealed oxidation status in CRF patients. The significantly elevated serum MDA concentration in CRF patients compared to controls reflects the increased formation of ROS and lipid peroxidation which is in accordance to Kim *et al.*, (2009). Meanwhile, CRF patient underwent dialysis with CVD showed higher mean of MDA levels than other groups. Similarly to our observation, Prakash *et al.*, (2010) also found dialysis patients were exposed to oxidant stress by the presence of free radical and had greater impairment compared to patients without dialysis. In recent years, the role of endothelial cell dysfunction and oxidative stress for development of CVD has been highlighted. The mechanisms involved free radical attacking on cell membrane polyunsaturated fatty acids results in formation of lipid peroxidation products such as MDA, which altered the function of endothelial cell and vascular smooth muscle (Sasikala *et al.*, 2000). The present study demonstrated a significant correlation between lipid peroxidation and oxidative stress in CRF patients (Annuk *et al.*, 2003). Hyperhomocysteinemia, abnormalities in lipid profile and lipid peroxidation are commonly found in patients with CRF which are recognized as risk factors for atherosclerosis (Dom *et al.*, 2003). Endothelium dysfunction and oxidative stress may be responsible for a metabolic block distally in transsulfuration route or at the homocysteine remethylation site causing hyperhomocysteinemia (Guldener, 2006; Guldener, 2005). Hyperhomocysteinemia, oxidative stress and endothelial dysfunction may be organized, forming a flow of atherothrombotic processes in CRF patients (Negane, 2009). Our data revealed that all CRF groups had significantly high level of homocysteine than control group with the highest concentration observed in CRF patient receiving dialysis with CVD compared to other groups, suggest patients incline towards experiencing a CVD event in ESRD (end stage renal disease) patients (Mallamaci *et al.*, 2002). Mallamaci and co-worker (2002) observed a clear association between high level of homocysteine and incident of cardiovascular mortality and atherothrombotic events in haemodialysis patients. In this regard, our study showed positive correlation between homocysteine, creatinine and MDA level, and was inversely correlated with HDL concentration. The pathologic mechanisms involved in atherosclerosis remain unclear but experimental data support a range of possibilities, including hyperhomocysteinemia, endothelial cell injury (Lim PS, 2001), enhanced LDL oxidation (Querfeld, 2001) increased thromboxane-mediated platelet aggregation (Mackman, 2008) and inhibition of the anticoagulant protein C and promotion of smooth muscle cell proliferation. Homocysteine interferes with the coagulation system and lead to prothrombotic effects. It is recognized 30-50% of pre-dialysis and dialysis patients have activated inflammatory response (Stenvinkel *et al.*, 2002). Our results showed that, CRF patients had significant high concentration of CRP compared with control groups. Our study showed significant relationship between CRP and increase risk of CVD in CRF patients. Oxidative stress may have association between endothelial dysfunction and chronic inflammation in CRF patients. Our data is in agreement with some other reports which have shown high CRP levels in CRF and dialysis patients predict a constant inflammatory state on follow up, that showed significant relationship between CRP and increase risk of CVD (Ortega *et al.*, 2001; Wong *et al.*, 2007). On the other hand, CRP is explained to play a role in multiple stages of atherosclerotic lesion development (Lacsan *et al.*, 2004). The mechanisms involved in CRP seems to be a direct mediator of endothelial dysfunction and inflammatory cell employment via the up-regulation of expression of endothelial cell adhesion molecules, by rising the release of monocyte chemotactic protein (MCP-1) (Devaraj *et al.*, 2004) and/ or endothelial derived contracting factors and by inhibiting the nitric oxide production (Jialal *et al.*, 2006). CRP promotes the uptake of low density lipoproteins (LDL) by foam cells

(Snyder *et al.*, 2005), which contributes to fibrous plaque formation and inhibits the remediable mechanism of angiogenesis (Rattazzi *et al.*, 2003).

#### 4.3 Evaluation of cohort study

In our cohort at Serdang Hospital, Malaysia the results showed 78.5% of deaths were among the patient with CVD. The results presented in this study were in agreement with numerous evident that patients with CRF have a substantially increased risk of CVD (Panza, 2009). The non traditional risk factors in this study such as hyperhomocystinemia, CRP, lipid peroxidation marker had strong association with the status of the CRF patients in our hospital which attribute the severity of the chronic renal failure of the patients. This finding may have important clinical consequences (Negane, 2009; Pawlak *et al.*, 2006). It is becoming evident that appropriate management of CVD in patients with CRF and dialysis may play a significant role in lowering the incidence of CVD morbidity and mortality in this population. Data collected from this study is hope to get more attention by the clinician in taking a proper measures in reducing the death toll of CRF patient that leading to CVD.

#### 5. Conclusion

In conclusion, the findings of this study indicated significant elevation in plasma MDA, CRP and homocysteine in CRF patients, with or without CVD. However, it appeared increased level of oxidative stress marker which was accompanied with hyperhomocysteinemia and lipid peroxidation. These factors may be important with respect to the high morbidity and mortality of CVD found in patients with CRF, probably through correlation with endothelial dysfunction. However, the current research is the first work in Malaysia, but prospective studies are needed to determine with certainty the degree of CRF and CVD associated with changes with respect to non-traditional risk factors.

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Table 1. Demographic and clinical characteristics of pre-dialysis and dialysis with and Without CVD versus the control

	Control	pre-dialysis		dialysis	
		with CVD	with CVD	with CVD	with CVD
Number	33	33	33	41	66
Age (years)	47.5±16.0	61.3±13.5 <sup>a</sup>	52.6±16.0	60.4±12.5 <sup>a</sup>	50.3±13.6
BMI (kg/m <sup>2</sup> )	23.7±3.3 <sup>Aa</sup>	24.8 ±3.7 <sup>Aa</sup>	28.3 ±4.9	23.6± 3.9 <sup>Aa</sup>	24.4 ±3.4 <sup>Aa</sup>
Waist circum (cm)	83.4± 9.8	89.2± 9.2	93.4± 9.9 <sup>a</sup>	86.7±9.8	86.4 ±10.1
Duration of CRF	-----	37.3±12.5	25.2 ±6.4	60.2±17.8 <sup>c</sup>	65.3±20.4 <sup>c</sup>
Smoking, %(n)	12(4)	15(5)	24(8)	26(11)	12(7)
Systolic BP(mmHg)	126.3±16.2	129.3± 26.2	147.6±26.3 <sup>b</sup>	147.3±25.1 <sup>b</sup>	140.0±24.3 <sup>b</sup>
Diastolic BP( mmHg)	78.7±11.2	70± 14.1 <sup>*</sup>	84.8±16.3	75± 11.1 <sup>*</sup>	78.3±12.2
<b>Druges</b>					
Antilipidemic, % (n)	-----	28(4)	33(11) <sup>*</sup>	29(12)	13.5(9)
Hypoglycaemic, % (n)	-----	70(23) <sup>**</sup>	33(11)	50(20)	13.5(9)
Antihypertension, % (n)	-----	50(15) <sup>***</sup>	52(16) <sup>***</sup>	39(16)	30(19)

**Note:** Data were presented using ANOVA post test (Tukey test) and t test, <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.001$  patients versus control and, <sup>Aa</sup> $p < 0.05$  pre-dialysis without CVD versus other groups, <sup>\*</sup> $p < 0.05$  pre-dialysis & dialysis with CVD versus. Pre-dialysis without CVD, <sup>\*\*</sup> $p < 0.05$  pre-dialysis with CVD versus other groups. <sup>\*\*\*</sup> $p < 0.05$  pre-dialysis groups versus dialysis groups. <sup>c</sup> $p < 0.05$  pre-dialysis without CVD versus dialysis groups.

Table 2. Biochemical parameters of pre-dialysis and dialysis, with or without CVD versus the control

Variables	Control	Pre- dialysis		dialysis	
		with CVD	without CVD	with CVD	without CVD
MDA (nmol/mL)	3.19±0.90	5.28±1.02 <sup>b,c</sup>	5.81±1.40 <sup>b</sup>	6.11± 1.71 <sup>b,c</sup>	5.40± 1.40 <sup>b</sup>
Homocysteine(μmol/L)	7.20±3.22	15.0±7.50 <sup>a</sup>	12.60±6.91 <sup>d</sup>	19.60±12.10 <sup>b,d</sup>	18.40±9.30 <sup>b,d</sup>
CRP (mg/L)	14.67±3.51	41.13±18.60 <sup>b,d</sup>	29.03±8.20 <sup>b</sup>	38.98±24.00 <sup>b,d</sup>	28.81±17.26 <sup>b</sup>
Cholesterol (mmol/L)	4.40±0.51	5.40±0.53 <sup>b</sup>	5.61±1.22 <sup>b</sup>	5.20± 1.21 <sup>a</sup>	5.10± 1.25 <sup>a</sup>
LDL-c (mmol/L)	2.60 ±0.51	2.90±0.80	3.10±1.00	3.00± 0.91	3.00± 1.10
Triglyceride (mmol/L)	1.05±0.38	2.32±0.92 <sup>b</sup>	2.02±1.04 <sup>b</sup>	2.23±1.56 <sup>b</sup>	2.02±1.04 <sup>b</sup>
HDL-c (mmol/L)	1.30±0.31	1.11±0.30	1.10±0.40	1.05± 0.33	1.10± 0.40

**Note:** Data were presented using ANOVA post test (Tukey test). <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.001$  patients versus control and, <sup>c</sup> $p < 0.05$  dialysis with CVD versus pre-dialysis with CVD, <sup>d</sup> $p < 0.05$  dialysis and pre-dialysis with CVD versus without CVD groups.

Table 3. Pearson correlation coefficients (r) of Homocysteine, CRP and MDA levels relative to another parameter

Variable	Correlation coefficient (r)		
	Homocysteine	CRP	MDA
Age	0.113	0.174 <sup>a</sup>	0.083 <sup>b</sup>
Creatinine	0.279 <sup>b</sup>	0.130 <sup>a</sup>	0.087
CRP	0.152	-----	0.157
MDA	0.276 <sup>b</sup>	0.157 <sup>a</sup>	-----
Homocysteine	-----	0.152	0.276 <sup>b</sup>
Cholesterol	0.097	0.155 <sup>b</sup>	0.058
LDL-c	0.111	0.039	0.012
HDL-c	-0.103	- 0.279 <sup>b</sup>	- 0.279 <sup>a</sup>
Triglyceride	0.092	0.413 <sup>b</sup>	0.413

**Note:** Bivariate correlation analysis was performed using SPSS software. Superscript letters a, b indicate that the correlation coefficient are statistically significant at the confidence levels of <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , respectively.

Table 4. Logistic regression analysis

Variables	OR(adjusted) Exp(β)	p	95% CI
Age	1.003	0.003	1.000-1.005
CRP	1.010	0.050	1.000-1.003
Cholesterol	1.211	0.020	1.101-1.301
Triglyceride	1.595	0.040	1.012-2.514
LDL-c	2.418	0.050	1.000-5.875

Note: The odds ratio for continuous variables are presented as the standardized regression coefficients as the term Exp (β); OR, odds ratio; CI, confidence interval.

Table 5. Follow up end point of CVD in CRF patients

	Pre-dialysis		dialysis CAPD		Haemodialysis	
	With CVD	without CVD	With CVD	without CVD	With CVD	without CVD
<b>MI (n)</b>	1	---	---	---	1	---
<b>Stroke(n)</b>	---	---	---	---	---	---
<b>Revascularization(n)</b>	---	---	---	---	1	---
<b>Dead (n)</b>	4	1	---	2	7	---

**Note:** Evaluation of end point of CVD in CRF patients with and without CVD in one whole year. Abbreviation: CAPD (peritoneal dialysis).