CARDIOVASCULAR RISK FACTORS ASSOCIATED WITH CHRONIC
RENAL INSUFFICIENCY IN BLACK PATIENTS AS SEEN
AT THE KENYATTA NATIONAL HOSPITAL.

A Dissertation submitted as part fulfilment of requirements for the
degree of Master of Medicine in Internal Medicine.

Dr. Nadeem I. Sheikh

University of Nairobi

2003
DECLARATION

I certify that this dissertation is my own original work and has not been presented for a degree at any other university.

Dr. N. I. Sheikh, MB, ChB (University of Nairobi)
This dissertation has been submitted with our approval as supervisors:

Dr. M. Joshi, MB, ChB, M.Med, MHH
Consultant Cardiologist and Lecturer,
Department of Medicine,
University of Nairobi.

Dr. J. K. Kayima, MB, ChB, M.Med
Consultant Nephrologist and Senior Lecturer,
Department of Medicine,
University of Nairobi.
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<th>Description</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>CRI</td>
<td>Chronic renal insufficiency</td>
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<tr>
<td>CRF</td>
<td>Chronic renal failure</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
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<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>HBA1c</td>
<td>Glycated haemoglobin</td>
</tr>
<tr>
<td>HDFP</td>
<td>Hypertension detection and follow-up programme</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta national hospital</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>Lipoprotein(a)</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>LVH</td>
<td>Left ventricular hypertrophy</td>
</tr>
<tr>
<td>MDRD</td>
<td>Modification of diet in renal disease study</td>
</tr>
<tr>
<td>NHANES III</td>
<td>Third national health and nutrition survey</td>
</tr>
<tr>
<td>NKF</td>
<td>National kidney foundation</td>
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<tr>
<td>PVD</td>
<td>Peripheral vascular disease</td>
</tr>
<tr>
<td>RRT</td>
<td>Renal replacement therapy</td>
</tr>
<tr>
<td>tHcy</td>
<td>Total plasma homocysteine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>USRDS</td>
<td>United States Renal Data System</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
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<tr>
<td>( \text{Waist Circumference} )</td>
<td></td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WHR</td>
<td>Waist Hip Ratio</td>
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</tbody>
</table>
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ACKNOWLEDGEMENT

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I am greatly indebted to, and wish to thank Dr. C. Maina and Dr. F. Rana for their critical assistance in providing the means for carrying out the echocardiograms and lab assays respectively.

The staff of the medical and renal clinics, Kenyatta National Hospital, who were of great help. I am also grateful to Dr. M. Twahir for his advice, Dr. A. Twahir and Roche products limited for their assistance in purchasing of several lab kits and use of their computer facilities, Aventis pharma for assistance in purchase of specimen bottles, syringes and gloves, the Lab manager and staff of M.P.Shah hospital for their assistance with regards to laboratory assays, my fellow registrars for their constant and unfailing support, Prof. D. Orinda for assistance with acquisition of the homocysteine assay kit, and all the patients and their relatives for accepting to be part of this study.

To my family I owe my undying gratitude for their unwavering support.
ABSTRACT

Background: Cardiovascular disease (CVD) is a major cause of morbidity and mortality among patients with chronic renal insufficiency (CRI). CVD is actually recognised as the leading cause of mortality and morbidity in the Chronic Renal Disease population. CVD mortality is approximately 10 to 30 times higher in patients treated by dialysis compared with patients in the general population, despite stratifying for gender, race, and the presence of Diabetes. After standardising for age, CVD mortality remains 10-fold higher in dialysis patients than in the general population, even at the extremes of age [1],[2]. The high prevalence of CVD in patients beginning dialysis suggests that CVD begins during or before the stage of CRI. Although there have been few studies of CVD in CRI, the available data suggest a higher incidence and prevalence of CVD than in the general population. No Data exists on the prevalence of cardiovascular risk factors in patients with chronic renal insufficiency at Kenyatta National Hospital.

Objectives: The aim of the study was to determine the prevalence of certain established and emerging cardiovascular risk factors, specifically, cigarette smoking, obesity, hypertension, dyslipidaemias, anaemia, hyperhomocysteinaemia, poor glycaemic control and left ventricular hypertrophy, in patients with Chronic Renal Insufficiency seen at the Kenyatta National Hospital.
**Design:** Cross-sectional prevalence study.

**Setting:** Tertiary Hospital (Kenyatta National Hospital), specialist nephrology clinic.

**Patient selection:** Consecutive sampling of CRI patients. CRI was defined as a calculated creatinine clearance of less than 75mls/min (documented twice at least 1 month apart, with no identifiable reversible cause. (Creatinine clearance was derived using the Cockcroft-Gault formula. [3])

**Variables of interest:** Age and sex, cigarette smoking, systemic arterial hypertension, obesity, anaemia, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, homocysteine levels, left ventricular hypertrophy, obesity, and poor glycaemic control among diabetic patients.

**Results:** Between April and October of 2002, 83 patients with chronic renal insufficiency were studied, 59 males and 24 females. The underlying aetiology of the Renal failure was Chronic glomerulonephritis in 36.1%, Hypertension in 31.3%, Diabetes mellitus in 28.9%, and polycystic kidney disease in 3.6%. The mean creatinine clearance was 47mls/min (95%CI 27.5-66.5mls/min) with 16.9% having a clearance less than 25mls/min, 34.9% having a clearance between 25 and 50mls/min, and 48% having a clearance between 50 and 75mls/min. 62.7% of the patients had age and sex as vascular risk factors. There were 6(7.23%) current smokers, all males. The mean BMI was 23.4kg/m² (95%CI 20.6-26.2), with 32.5% of patients being either overweight or obese. 15 of the 27 obese patients had central obesity. 61.5% of patients were hypertensive, of these patients, 81% were on anti-hypertensive
treatment. 4.8% of patients had elevated total cholesterol levels. 12% of patients had elevated LDL-Cholesterol levels. 73 (88%) patients had low HDL-cholesterol levels. 13 (15.7%) patients had elevated triglyceride levels. The mean Homocysteine level was 24.1 Dmol/l (95% CI 10.2-38). 77 patients (92.8%) had elevated homocysteine levels. The mean glycated haemoglobin level among the 24 diabetic patients was 7.43 (95% CI 6-8.8), with 37.5% of patients exhibiting poor glycaemic control. 72.3% of patients had elevated blood pressures, of these patients, 81% were on anti-hypertensive therapy and demonstrated less than optimal control. The mean haemoglobin was 11.6 g/dl (95% CI 9.1-14). 49 patients (59%) of patients were anaemic. Significantly, creatinine clearance was inversely co-related to haemoglobin levels (p = .01212). Male patients were more likely to be anaemic than female patients (p = .00001) there was also a significant relationship between level of blood pressure and haemoglobin level (p = .00050). The mean left ventricular mass index was 133.6 g/m$^2$ (95% CI 94.3-172). Males were more likely to have high left ventricular mass indices (p = .03800). There was a significant inverse relationship between creatinine clearance and left ventricular mass index (p = .00047) Similarly, there was a significant inverse co-relation of haemoglobin level and left ventricular mass index (p < .00001). There was a strong relationship between level of blood pressure and left ventricular mass index (p = .00003). Multiple regression analysis revealed that the relationships of Haemoglobin level and Hypertension to left ventricular hypertrophy were independent of other variables. (p < 0.0001 in both cases). 94% of patients had two or more cardiovascular risk factors.
Conclusions: There is a high prevalence of cardiovascular risk factors, frequently multiple, in patients with chronic renal insufficiency seen at KNH. A significant relationship between Anaemia, hypertension and left ventricular mass index was also demonstrated.
1. LITERATURE REVIEW

The Chronic Renal Insufficiency population is a large group of patients who have largely gone unnoticed by the research community for several reasons, but who have now come to importance as it is now recognised that their effective management can significantly reduce future morbidity and mortality for these patients and afford them a richer quality of life.

CRI refers to that group of patients who have begun to show a decline in renal function but who are still able to function without renal replacement therapy. The effective management of these patients has already begun to show good results in institutions where their specific requirement are recognised and met.

Cardiovascular disease (CVD) is a major cause of morbidity and mortality among patients with chronic renal insufficiency (CRI). The prevalence of coronary disease among haemodialysis patients in the United States is approximately 40%. Irrespective of diabetic status, patients with CRI have cardiovascular mortality rates at least 10 times greater than the general population. Although the prevalence of CVD and cardiac risk factors (such as hyperlipidaemia, hypertension, and diabetes mellitus) among dialysis patients is well described, less is known about patients with CRI who do not require dialysis.
Patients with end-stage renal disease are at high risk for developing premature vascular disease.\(^1\) In the United States, cardiac mortality for dialysis patients aged younger than 45 years is more than 100 times greater than that in the general population\(^{[1]}\). However, little is known about its evolution in relation to more minor degrees of renal impairment. In the general population, mild renal impairment is associated with increased risk for coronary artery disease (CAD) and stroke,\(^6\) suggesting that cardiovascular disease begins to develop early in the natural history of chronic renal insufficiency.\(^6\) For example, in one community survey of healthy middle-aged men, elevated serum creatinine concentration (—1.47 mg/dL) was associated with age-adjusted relative risks of 1.8 for cardiovascular death, 1.5 for CAD, and 3.0 for stroke.\(^7\)

1.1 A REVIEW OF CVD IN CRI

**Spectrum of Disease**

Cardiovascular mortality rates among patients with ESRD are significantly higher than are those among the-general population, even when adjustments are made for age, gender, and diabetic status. Cardiovascular mortality rates in transplant recipients, a model of gradual CRI, appear to fall within the ranges seen in the general population and those seen in dialysis patients.
The prevalence of both ischaemic heart disease and cardiac failure is approximately 50% among patients starting renal replacement therapy in the United States (Table 1). Approximately 80% of patients start dialysis therapy with LVH, left ventricular dilatation, or systolic dysfunction. The prevalence, incidence, and prognosis of clinically manifested CVD are not known with precision in CRI patients but are highly likely to exceed those seen in the general population.\[8\] Left ventricular enlargement is common in CRI, with an inverse relationship between the prevalence of LVH and kidney function.\[9\]\[10\] The abnormalities are reversed by transplantation, but only partially, suggesting that the duration of exposure to known and unknown risk factors may be as important in pathogenesis as the levels of these risk factors.\[12\] Risk factor prevalence has been noted to be significant in the CRI population: (13)
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<tr>
<td>1986-87</td>
<td>3,399</td>
<td>3,468</td>
<td>24,497</td>
<td>5602</td>
<td>433</td>
</tr>
<tr>
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<table>
<thead>
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<td>1986-87</td>
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<table>
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<tr>
<th>Coronary artery disease</th>
<th>All</th>
<th>40.8%</th>
<th>36% (PD)'</th>
<th>37.2%</th>
<th>14.1%</th>
</tr>
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</tbody>
</table>

| Myocardial infarction   |     |       |            |       |       |
| Diabetic < 45 yrs       |     |       |            |       |       |
| Diabetic > 45 yrs       |     |       |            |       |       |
| Non-diabetic < 45 yrs   |     |       |            |       |       |
| Non-diabetic > 45 yrs   |     |       |            |       |       |
| Angina                  |     |       |            |       |       |
| All                     |     |       |            |       |       |
|                           |     |       |            |       |       |
| Congestive heart failure | All | 41.1% | 31% (PD) | 30.8% |       |
|                           |     |       | 40% (HD)  |       |       |
| left ventricular hypertrophy | All | 30.9% | 18% (PD) | 74.0% |       |
|                           |     |       | 22% (HD)  |       |       |
| Cardiomegaly            | All | 38.1% |           |       |       |

Abbreviations: PD, peritoneal dialysis; HD, haemodialysis.
Left ventricular remodelling is a complex process whose biological basis is beginning to be unravelled at the molecular level. The sensor and effector mechanisms are not yet understood in humans, but genetic, hormonal, and metabolic factors are likely to be important.\textsuperscript{[18]} Cardiac myocyte enlargement is usually accompanied by increased rates of fibrous tissue deposition and myocyte apoptosis in animal models.\textsuperscript{181} Metabolic factors, including the uraemic microenvironment and excessive local angiotensin II, endothelin, bradykinin, adrenergic, and parathyroid activity, have been suggested as being aetiological in animal models of uraemic cardiomyopathy.\textsuperscript{191} The influence of classic haemodynamic factors, such as hypertension and anaemia, has been modest or absent in animal studies. This is in direct contrast to human uraemia, in which these haemodynamic factors have been dominant, in terms of both associative power and consistency across studies.

For example, pressure overload due to aortic stenosis or hypertension mandates an augmentation of systolic intraventricular pressure. This is achieved by arraying myocytes in parallel, with an increase in ventricular wall thickness and a higher ratio of wall thickness to cavity diameter. This process, known as concentric LVH, results in enhanced ventricular stiffness, or diastolic dysfunction, and increased perfusion requirements, especially during exercise. Concentric LVH is common in patients with CRI, with a prevalence of approximately 40% in those starting dialysis therapy.[1]
Hypertension appears to be the principal modifiable risk factor for concentric LVH. Another example is volume overload. Whether is it caused by aortic incompetence, the peripheral vasodilatation that occurs with anaemia, or an arterio-venous fistula, volume overload mandates an augmentation of cardiac output typically by increasing both the heart rate and the stroke volume of the heart. Lengthening of cardiac myocytes is accompanied by increased cardiac output, according to Starling's Law of the Heart. Left ventricular dilatation ensues, so that the ratio of ventricular wall thickness to cavity diameter stays the same or decreases. Laplace's law, applied to a hollow spherical object, states that wall tension is directly proportional to both diameter and intracavitary pressure and indirectly proportional to wall thickness. In isolation, left ventricular dilatation leads to augmented wall tension, which is inherently unfavourable because metabolic fuel requirements and oxygen consumption are greater. Ventricular wall thickening is a useful second-order response in this situation, tending to lower wall tension. Left ventricular dilatation is common in CRI patients and appears to be the most characteristic morphology among uraemic patients, in the absence of hypertension and coronary artery disease. Anaemia appears to be an important, modifiable risk factor for this process in CRI patients.
Anaemia has a number of deleterious effects, both direct and indirect, on the cardiovascular system. In both dialysis and CRI populations, anaemia has been identified consistently as an independent risk factor for LVH and for hospitalisations related to cardiac and non-cardiac causes.\(^{(11)}\)\(^{(23)}\)\(^{(24)}\) Anaemia leads to an increase in cardiac workload due to relative tissue ischaemia, which in turn leads to development of LVH. Sustained anaemia often leads to eccentric LVH, in contrast to the concentric LVH associated with sustained hypertension or pressure overload. Studies in animals have linked haemoglobin level and hypoxia directly to left ventricular growth, hypothetical^ caused by triggering of angiotensin systems and attendant rises in levels of angiotensin II, a well-characterized myocyte growth factor.\(^{(11)}\)\(^{(11)}\)\(^{(11)}\)\(^{(11)}\)

Two study groups have investigated the onset, or changes in symptoms, of CHF in incident dialysis patients and in a cohort of patients with CRI.\(^{(11)}\)\(^{(20)}\) In both observational cohort studies, the independent effect of anaemia on the development of CHF was clear.

Historically, most studies have defined anaemia a priori using threshold values of haemoglobin less than 10 g/dL or a haematocrit less than 28%. More recently it has been determined that even at higher absolute levels of haemoglobin, a relative decrease from baseline can be associated with left ventricular growth, thus providing strong evidence that earlier attention to changes in haemoglobin values in CRI patients is important.\(^{(24)}\)\(^{(25)}\) Further corroboration of this concept can be obtained from recent studies such as that by Hayashi's group, in which regression of LVH was achieved after
haemoglobin levels were corrected to values above those currently recommended for dialysis populations. The relative risk for an increase in left ventricular mass has been reproducibly estimated to be in the range of 1.32 to 1.60 for each gram of haemoglobin decrease, indicating again the powerful association of anaemia on patient outcomes.

In an intriguing recent observation by Silverberg et al, 26 patients with a history of refractory class IV CHF who were identified as being "relatively" anaemic (mean entry haemoglobin of 10.16 ± 0.95 g/dL) were treated with erythropoietin and iron. Treatment of the relative anaemia in this fashion achieved a mean haemoglobin of 12.10 ± 1.21 g/dL and resulted in major reductions in symptoms, hospital visits, and inpatient admissions relative to baseline. It is noteworthy that the 26 patients who were treated prospectively had a mean entry serum creatinine value of 2.59 ± 0.77 mg/dL, signifying a moderate degree of kidney disease. Although the sample size was small and the trial was not randomized or controlled, it emphasizes the importance of recognizing and aggressively managing anaemia in patients with CRI and CVD.
<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical Features</th>
<th>Prevalence</th>
<th>Outcomes</th>
<th>US Annual Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>End stage</td>
<td>ESRD</td>
<td>335,000</td>
<td>CVD, death</td>
<td>&gt;$14 billion</td>
</tr>
<tr>
<td>Progression</td>
<td>Chronic renal insufficiency (Decreased glomerular filtration rate or increased serum creatinine)</td>
<td>10.9 million</td>
<td>ESRD, CVD, death</td>
<td></td>
</tr>
<tr>
<td>Initiation</td>
<td>Albuminuria, proteinuria</td>
<td>30 million</td>
<td>Chronic renal insufficiency, CVD, death</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>Elderly, ethnic minorities, hypertension, diabetes, autoimmune disease, genetic diseases, and family history</td>
<td>30 million</td>
<td>Chronic renal insufficiency, CVD, death</td>
<td>7 billion</td>
</tr>
</tbody>
</table>
1.4.1 End Stage

End-stage renal disease (ESRD), defined as a life-threatening reduction in renal function, requiring dialysis or transplantation to survive, is the most visible consequence of CRD (Table 2).

Data from the US Renal Data System (USRDS) from 1998 indicate 335,000 patients with ESRD. From 40% to 75% of patients starting dialysis already have manifestations of CVD, and CVD accounts for 40% of deaths. Patients treated by dialysis suffer a high burden of co-morbid conditions, reduced quality of life, and an annual mortality rate of approximately 25%.

Treatment costs for ESRD exceed 14 billion dollars per year in the USA. Reduction of CVD mortality and morbidity in ESRD will require more effective treatment of CVD, as well as prevention of CVD through risk factor reduction during earlier stages of CRD.

1.4.2 Progression

ESRD is preceded by a stage of progressive decline in renal function, known as chronic renal insufficiency (CRI). The earliest manifestation of this stage is a reduction in the glomerular filtration rate (GFR) below the normal range and subsequently an increased serum creatinine level.
Data from the Third National Health and Nutrition Survey (NHANES III) suggest there are approximately 10.9, 3.0, and 0.8 million people in the United States with serum creatinine concentrations -1.5, 1.7, and 2.0 mg/dL, respectively.\textsuperscript{1271}

The risk of progression to ESRD depends on many risk factors, including whether CRI is due to CRD or haemodynamic factors associated with other diseases (pre-renal factors), level of GFR, rate of decline in GFR, and age. In principle, the lifetime risk of developing ESRD would be greater in younger versus older patients, patients with versus without CRD, patients with lower versus higher baseline GFR when CRI is detected, and patients with faster versus slower subsequent GFR decline. As emphasized later, patients in this stage are at risk for the development of CVD as well as progression to ESRD. The cost to the United States of taking care of this population remains unknown.

\textbf{1.4.3 Initiation}

In principle, we can consider the initiation of CRD to begin with an unresolved renal injury. Few clinical markers are sensitive or specific for renal injury or the initiation of CRD. The best marker at our disposal is the presence of albuminuria or proteinuria. Small amounts of either albumin or protein in the urine are important early signs of CRD, as well as strong predictors of an increased risk of CVD morbidity and mortality in certain high-risk groups.

The prevalence of dipstick-positive proteinuria was approximately 4\% in both the Framingham Heart Study and the Okinawa Mass Health Screening
presumably more sensitive measures of CRD initiation would identify a much higher number of individuals affected. The NKF conference "Proteinuria, Albuminuria, Risk, Assessment, Detection, and Elimination (PARADE)" evaluated the testing and quantification of urinary albumin and protein the relationship of proteinuria to CVD and to progressive renal disease the relationship of the nephrotic syndrome to proteinuria, and the appropriate patterns of nephrologic referral in patients with proteinuria or albuminuria. It concluded that patients with proteinuria or albuminuria are at risk for the development of progressive renal disease and CVD. The cost of care for patients with proteinuria or albuminuria remains unknown.

1.4.4 At-Risk Population

The populations at risk for CRD are the elderly, and those with diabetes, hypertension, autoimmune diseases, recognized genetic diseases, or a family history of renal disease. Ethnic minorities in the USA, such as African Americans, Hispanics, and Native Americans, appear particularly susceptible to the development of CRD. Many of these same subgroups are at risk factors for CVD. Current US estimates of patients with diabetes and hypertension are 8 (diagnosed cases) and 50 million, respectively. The elderly and ethnic minorities are the fastest growing segments of the US population, by implication these problems would be grossly manifest in countries such as ours. Therefore, the number of people at risk for CVD is potentially enormous. The cost of caring for this large group of patients is unknown. Diabetes and Hypertension are growing problems in the Kenyan
and hence the number of people at risk for CVD is increasing concomitantly.

1 5 GENERAL OVERVIEW OF EPIDEMIOLOGY OF CVD IN CRD

1.5.1 CVD in ESRD

CVD mortality is approximately 10 to 30 times higher in patients treated by dialysis compared with patients in the general population, despite stratifying for gender, race, and the presence of diabetes (Table 3).\(^1\) After stratifying for age, CVD mortality remains 10-fold higher in dialysis patients than in the general population, even at the extremes of age. Renal transplant recipients are also at high risk for CVD mortality. Data from the USRDS suggest that the annual death rate from CVD is approximately twofold higher (Table 3).\(^{15}\) This is likely an underestimate of the true mortality risk because of incomplete ascertainment of cause of death in patients who received transplants. Even after stratifying for age, gender, race, and the presence of diabetes, the CVD mortality rate in transplant recipients remains higher than in the general population. In principle, the high CVD mortality rate in ESRD could be due to a high case fatality rate or a high prevalence of CVD. In fact, both are true.
TABLE 3 Comparison of CVD Mortality in the General Population and ESRD by Gender, Race, and Presence of Diabetes (Annual Mortality, %)

<table>
<thead>
<tr>
<th>Gender</th>
<th>All General Population (%)</th>
<th>Men</th>
<th>Women</th>
<th>White</th>
<th>Black</th>
<th>Diabetic</th>
<th>Nondiabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.28</td>
<td>0.28</td>
<td>0.27</td>
<td>10.29</td>
<td>0.23</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.28</td>
<td>10.53</td>
<td>0.56</td>
<td>1.11</td>
<td>0.59</td>
<td>0.39</td>
</tr>
<tr>
<td>Haemodialysis</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Peritoneal dialysis</td>
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</tr>
<tr>
<td>Renal transplant recipient</td>
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</table>

NOTE. CVD mortality is defined as death due to arrhythmias, cardiomyopathy, cardiac arrest, myocardial infarction, atherosclerotic heart disease, and pulmonary oedema.

'General population (GP). Data, from National Centre for Health Statistics 1993 multiple cause of mortality data files (n = 2.27 million deaths). ICD-9 codes 402, 404, 410-414, and 425-429. Diabetes in GP was defined by ICD-9 code 2500-2509 mentioned anywhere on the death certificate. Diabetes in GP was calculated using multiple causes of mortality data files, population.
CVD mortality is underestimated in diabetic individuals and overestimated in diabetic individuals due to incomplete listing of diabetes on death certificates.

T ESRD patients treated by haemodialysis, peritoneal dialysis, and renal transplantation (n = 50,227 deaths). Data from USRDS 1994-1996 (special data request) HCFA form 2746 no. 23, 26-29, and 31. Death rates per 1000 patient-years at risk were converted to annual percent mortality. CVD mortality is underestimated in renal transplant recipients because of incomplete ascertainment of the cause of death.

The case fatality rate of CVD in dialysis patients is extremely high. Using data from the USRDS, Herzog et al evaluated 34,189 patients on long-term dialysis and found that mortality rates 1 year and 5 years after myocardial infarction were 59% and 90%, \(^{33}\) respectively, which is much higher than the rate after myocardial infarction in the general population, even in patients with co-morbid conditions. For example, in the Worcester Heart Attack Study during the period of 1975 to 1986, 2-year mortality rates after myocardial infarction in diabetic men and women were 25% and 34%, respectively. \(^{34}\) A high case fatality rate in dialysis patients is also observed in patients with congestive heart failure (CHF). For example, in a Canadian cohort study, the median survival duration was only 18 months after development of de novo CHF. \(^{35}\)
Data from the UKKD study in 1997 show a high prevalence of CVD in incident and prevalent dialysis patients (Table 4).\cite{15} Using clinical criteria, coronary artery disease (CAD) and CHF were each present in 40% of an incident dialysis population. Although the prevalence of CAD is high, this appears to be an underestimation of the true prevalence of CAD, as shown in angiographic studies of asymptomatic diabetic patients.\cite{36} Foley et al demonstrated that approximately 75% of an incident dialysis cohort had left ventricular hypertrophy (LVH) by echocardiography.\cite{17}

**TABLE 4. Approximate Prevalence (%) of CVD in the General Population and Dialysis Patients**

<table>
<thead>
<tr>
<th>Population</th>
<th>Left Ventricular Coronary Artery Hypertrophy (clinical)</th>
<th>Left Ventricular Hypertrophy (echo)</th>
<th>Congestive Heart Failure (clinical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population</td>
<td>5-12\textsuperscript{1}</td>
<td></td>
<td>5 \textsuperscript{o}</td>
</tr>
<tr>
<td>Chronic renal insufficiency</td>
<td>NA</td>
<td>25-50 (varies with renal function)\textsuperscript{5}</td>
<td>NA</td>
</tr>
<tr>
<td>Haemodialysis</td>
<td>42</td>
<td>\textasciitilde 75\textsuperscript{11}</td>
<td>40</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>40</td>
<td>\textasciitilde 75\textsuperscript{11}</td>
<td>40 \textsuperscript{o}</td>
</tr>
<tr>
<td>Renal transplant</td>
<td>15\textsuperscript{1}</td>
<td>\textgammachar 60 \textsuperscript{60}</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.
Lower value, age 45-64 years; higher value, age <65 years. Data from National Heart Lung and Blood Institute (NHLBI) Morbidity and Mortality Chart book 1996.\textsuperscript{371} Data from Levy et al.\textsuperscript{38} Age 60 years. Data from NHLBI Morbidity and Mortality Chart book 1996.\textsuperscript{371} Data from Levin et al.\textsuperscript{10} Data from Dialysis Morbidity and Mortality (Wave 2). USRDS Annual Data Report, 1997.\textsuperscript{1391} Data from Foley et al.\textsuperscript{7} Data from Kasiske.\textsuperscript{1401} Data from Parfrey et al.\textsuperscript{41}

### 1.5.2 CVD IN CRI

It became apparent in the early 1970s that cardiovascular mortality rates among ESRD patients are exceptionally high. Accelerated atherosclerosis, caused by the uraemic state, was believed by many to explain these findings. More recently, the prognostic primacy of cardiac failure has become more apparent. The vast majority of studies to date suggest that cardiac failure portends early demise among dialysis patients. In one study, cardiac failure at inception of dialysis therapy was associated with a 2-fold increase in mortality.
chocardiographic left ventricular mass index is strongly associated with cardiovascular mortality in the general population, an association replicated in dialysis patients in a 1989 study. Left ventricular cavity volume is also strongly associated with mortality among dialysis patients, and overall geofunctional classification (eg, normal, concentric LVH, left ventricular dilatation with normal systolic function, systolic dysfunction) has a robust association with future ischaemic heart disease, cardiac failure, and death among dialysis patients.

Regression of LVH lessens the likelihood of cardiac failure in uraemic patients, suggesting that echocardiographically detected abnormalities are an intermediate stage between cost-free physiologic adaptation and end-stage maladaptation. This suggests that one focus of early intervention should be on measures that prevent cardiac hypertrophy in CRI patients.

The high prevalence of CVD in incident dialysis populations suggests that CVD begins during or before the stage of CRI. Although there have been few studies of CVD in CRI, the available data suggest a higher incidence and prevalence of CVD than in the general population. Levin et al showed that the prevalence of LVH is inversely related to the level of renal function. In a cross-sectional study, the prevalence of LVH by echocardiography was 27%, 31%, and 45% in patients with creatinine clearance greater than 50, 25 to 50, and less than 25 mL/min, respectively. Anaemia and hypertension were risk factors for the presence of LVH. The high prevalence of LVH in CRI contrasts with...
ha ly with a prevalence of less than 20% in patients of similar age in the general population.\textsuperscript{[42]} A more recent prospective study of CRI by Levin et al documented increasing left ventricular mass in association with rising blood pressure and declining haematocrit.\textsuperscript{[11]} Other studies have shown that partial correction of anaemia\textsuperscript{[43]} and treatment of hypertension lead to regression of LVH in CRD. Angiotensin-converting enzyme inhibitors have been shown to reduce LVH in addition to their effects on blood pressure.\textsuperscript{[44]} However, it remains to be determined whether regression of LVH, independent of blood pressure reduction, reduces CVD morbidity or mortality in either the general population or CRD patients.

Most studies of CAD and CHF in CRI have been cross-sectional, making it difficult to interpret cause-and-effect relationships. That is, it is difficult to determine whether CVD leads to CRI, or whether the CRI leads to the development of CVD. These questions can only be answered by prospective studies. One such study of CRI that specifically focused on CAD outcomes assessed the incidence of first myocardial infarction and stroke in 147 patients.\textsuperscript{[45]} The incidence of myocardial infarction in males of all ages and females until the age of 65 was approximately two to three times higher in CRI patients than in the general population. Risk factors for these events included smoking, hypertension, increased fibrinogen levels, hyperhomocysteinemia, and lower high-density lipoprotein (HDL) cholesterol levels. Because the study was small, it may not have had sufficient statistical power to evaluate other risk factors.
Additional support for the suggestion of a higher risk of CVD in CRI patients than in the general population derives from prospective studies showing a lower level of renal function is an independent predictor of all-cause and CVD mortality. The Hypertension, Detection, and Follow-up Program (HDFP) was a randomised controlled trial of 10,940 patients designed to evaluate the effect of different levels of blood pressure control on all-cause and CVD morbidity and mortality. An ancillary analysis was performed to evaluate the association of baseline serum creatinine with these outcomes. The results indicated that patients with baseline creatinine levels —1.7 mg/dL had an 8-year mortality rate three times higher than those with creatinine values less than 1.7 mg/dL. In multiple logistic regression analysis, the relative odds ratio for death was 2.2 in the group with the higher baseline creatinine level. Of note was that CVD mortality in the subgroup with higher serum creatinine levels was three times higher than mortality due to renal failure. The latter is important from a practical standpoint, as well as in the design of prospective studies. Patients with CRI may be at higher risk for CVD than ESRD.

The Cardiovascular Health Study was a prospective, observational study designed to determine the risk factors for and the consequences of CVD in 5,201 older adults in the general population. Fried et al used a Cox proportional hazards analysis to evaluate risk factors for mortality over a mean follow-up period of 4.25 years. Twenty characteristics, one of which was higher baseline serum creatinine, were significantly and independently associated with mortality. Patients with a baseline serum creatinine level —1 5 mg/dL had a relative risk of 1.71 for death, compared with patients with a baseline serum creatinine of less than 1.5 mg/dL.
reasons for the independent effect of level of renal function on CVD mortality and morbidity, as well as on all-cause mortality, remain unknown. One explanation is that a decreased level of renal function is associated with risk factors that were not measured in these multivariable analyses. Another explanation is that a decreased level of renal function is associated with subclinical CVD at baseline. The decreased level of renal function would therefore be a marker of CVD.

1.6 EPIDEMIOLOGY OF CVD RISK FACTORS IN CRD

CVD risk factors in CRD can be divided into traditional and non-traditional factors. For convenience, we define traditional coronary risk factors as those derived from studies of the Framingham population, such as older age, diabetes, male gender, family history of coronary disease, hypertension, high low-density lipoprotein (LDL) cholesterol, low HDL cholesterol, history of smoking, physical inactivity, menopause, and psychosocial stress. We define non-traditional risk factors as other CVD risk factors that increase in prevalence or severity as renal function declines. These include, among others, albuminuria or proteinuria, extracellular fluid volume overload, electrolyte imbalance, hypertriglyceridaemia, elevated lipoprotein (a) [Lp (a)], hyperhomocysteinaemia, markers of inflammation or infection (eg, C-reactive protein [CRP]), increased oxidant stress, thrombogenic factors, malnutrition, anaemia, and other uraemic toxins. The NKF Task Force Report and a recent issue of Seminars in Dialysis provide a more thorough discussion of traditional
and non-traditional (sometimes referred to as uraemia-related) risk factors, as well as treatment recommendations. [4] [48]

1 6.1 Traditional CVD Risk Factors

Furth et al evaluated the relationship of traditional CVD risk factors to mortality in incident dialysis patients using data from the Case-Mix Severity Study of the USRDS. [49] As in the general population, their analysis showed that white race, older age, male gender, diabetes, and smoking were independent risk factors for death. Unlike the general population, higher serum cholesterol and higher systolic blood pressure were not risk factors for mortality.

The prevalence of hypertension is approximately 80% in dialysis patients. [50] However, the relationship between hypertension and mortality is U-shaped. [51] High blood pressure in dialysis patients has now clearly been shown to be a risk factor for the development of LVH, CAD, and CHF. [52] It is likely that the development of these conditions is followed by a decline in blood pressure, and we suspect that the higher mortality rate observed in patients with low blood pressure most likely reflects confounding by CVD, especially CHF.

The prevalence of increased total cholesterol and LDL cholesterol is not increased in dialysis patients compared with the general population. [53] However, HDL cholesterol levels tend to be lower than in the general population. The relationship of total serum cholesterol to mortality is also U-shaped. [54] The increased mortality risk at low serum cholesterol levels most likely reflects confounding by malnutrition, as serum cholesterol and serum
lb min concentrations are inversely related in this population. Thus, it is unclear whether elevated total cholesterol is a risk factor for CVD in dialysis patients.

Several studies have attempted to determine whether the burden of traditional risk factors in CRD is sufficient to account for the higher CVD morbidity and mortality. Coronado et al examined traditional coronary risk factors in an ancillary analysis of 1,795 patients with CRI enrolled in the Modification of Diet in Renal Disease (MDRD) study. They computed the coronary point score, an aggregate measure of traditional coronary risk factors derived from the Framingham population, which predicts the probability of developing CAD over 5 to 10 years in individuals initially free of CAD. The coronary point score in MDRD study participants was weakly, although significantly, negatively correlated with baseline GFR in both men and women ($r = -0.09$, $P = 0.01$ and $r = -0.21$, $P > 0.001$, respectively). This is consistent with the hypothesis that coronary risk factors are acquired or worsen as GFR declines in CRI. However, the coronary point score, even extrapolated to a GFR of 0 mL/min (equivalent to ESRD), was not substantially greater than that of individuals of similar age in the general population. Cheung et al examined the levels of traditional coronary risk factors in prevalent haemodialysis patients enrolled in the Haemodialysis (HEMO) study. The risk of developing de novo CAD, as predicted by the coronary point score, was similar in HEMO study participants to individuals of similar age in the general population. The lower predicted incidence of de novo CAD contrasts sharply with the high observed incidence in dialysis patients. These studies suggest that the burden of traditional risk factors may not be sufficient to account for the higher
and morbidity of CVD in CRD. It is hypothesized that other risk factors, e.irh as uraemia-related risk factors, are likely also important, uraemia-related risk factors, are likely also important,

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^on-traditional Risk Factors

Extracellular fluid volume overload and electrolyte imbalance.

Fluid and electrolyte abnormalities are extremely common in CRD, but there is little data available on their role in the pathogenesis of CVD. Abnormalities of calcium, phosphorous, and parathyroid hormone metabolism are associated with CVD morbidity and mortality, while shifts in electrolytes, in particular potassium, have been hypothesized to contribute to sudden death in dialysis patients. Haemodynamic overload is associated with LVH, non atherosclerotic enlargement, and hypertrophy of large conduit arteries, as well as occlusive atherosclerotic lesions of smaller vessels. These alterations are important in the pathogenesis of CVD in CRD.

B. Hypertriglyceridaemia.

The role of hypertriglyceridaemia as a risk factor for atherosclerosis remains elusive. Although high triglyceride levels are generally predictive of CVD risk in the general population, multivariate adjustment for other risk factors, especially high LDL cholesterol and low HDL cholesterol, diminishes this association. Nevertheless, a growing body of evidence suggests that hypertriglyceridaemia is an independent risk factor for CVD in the general population. The prevalence of hypertriglyceridaemia is especially high in Patients treated by haemodialysis or peritoneal dialysis.
c. **Lp(a)**

Lp(a) is similar to LDL cholesterol, with the addition of a large glycoprotein, designated as apolipoprotein(a). Elevated Lp(a) levels have been found to be independent predictors of CVD in most, although not all, studies in the general population.\(^{1601}\) LP (a) levels are known to increase in CRI patients and in patients treated by haemodialysis and peritoneal dialysis.\(^{1621}\) Both cross-sectional and prospective studies in ESRD have shown associations between higher Lp(a) levels and CVD\(^{1651}\); however, these findings have not been consistent.\(^{1661}\) It is also unclear whether the lipoprotein phenotype or the Lp(a) level is more predictive of CVD events.

D. **Homocysteine.**

Elevated total plasma homocysteine (tHcy) levels greater than 14 mumol/L occur in approximately 90% in patients with ESRD,\(^{67}\) as compared with 5% of the general population and approximately 35% of the general population with CAD.\(^{68}\) There are now convincing data that mild to moderate increased tHcy is an independent risk factor for CVD in the general population.\(^{69}\) Although not conclusive, retrospective and, more recently, prospective studies have suggested that increased tHcy is associated with CVD outcomes in ESRD as well.\(^{70}\)\(^{71}\) Although low doses of folic acid (400 mug/d) are successful in lowering homocysteine levels in the general population, doses as high as 15 mg/d for 8 weeks provided only a 26% reduction in tHcy levels in dialysis patients.\(^{72}\) Furthermore, because homocysteine levels are so high in the dialysis population, the levels were lowered to less than 15 mumol/L in only 33% of patients.
Inflammation is now believed to play a significant role in the pathogenesis of atherosclerosis in the general population. In the Physicians Health Study, CRP level was found to be an independent predictor of myocardial infarction and stroke. It was also noted that the reduction in the risk of myocardial infarction associated with the use of aspirin was directly related to the level of CRP raising the possibility that anti-inflammatory agents may have benefits in reducing CVD. CRP levels are known to be elevated in both ESRD and in predialysis patients, and elevated levels of CRP are associated with higher all-cause mortality, as well as CVD mortality. Cross-sectional analysis has also shown higher levels of IgA reactive to Chlamydial pneumonia in predialysis patients with the higher CRP levels, suggesting a possible causal relationship between Chlamydia pneumonia infection and CAD. There have been no studies in CRD of the effects of anti-inflammatory or antimicrobial agents on CRP, levels of other markers, or the incidence of CVD.

F. Thrombogenic factors.

Thrombogenic factors appear to be risk factors for CVD in the general population. For example, the highest versus the lowest tertile of fibrinogen levels was associated with an odds ratio for CAD of 2.3 in a large pooled analysis. Fibrinogen levels also appear to be associated with angiographic severity of disease, recurrent ischaemic events, and risk for restenosis after
Cross-sectional studies suggest that fibrinogen levels are higher in patients treated by haemodialysis or peritoneal dialysis, as well in the CRI population compared with the general population. Among patients with CRD, haemodialysis patients have higher levels of platelet aggregation, while those on peritoneal dialysis have lower levels of tissue plasminogen activator and higher levels of fibrinogen and factor VII. Koch et al showed an association between fibrinogen levels and CAD in haemodialysis patients in cross-sectional analysis.

G. Oxidative stress.

Oxidative modification of LDL cholesterol plays a central role in atherogenesis. Observational studies in the general population have suggested that high intakes of antioxidants such as vitamins E, C, and A may provide protection against CVD. Oxidant stress is higher in patients with CRD compared with the general population, likely due to both low-grade inflammation and to reduced antioxidant substances.

H. Anaemia.

The existence of anaemia in CRD is almost universal. Anaemia is a risk factor both for the development of de novo and recurrent CHF, as well as for CVD mortality. It is also one of the important risk factors for the development of LVH. Partial correction of anaemia by erythropoietin therapy reduces LVH in CRI patients, as well as in dialysis patients. Complete correction of anaemia did not improve outcomes in one study of dialysis patients with pre-
existing CVD. It remains to be determined whether complete or partial correction of anaemia in earlier stages of CRD will decrease CVD morbidity or mortality.

/ Uraemic toxins.

Other uraemic toxins and dialysis-related factors are likely important in the promotion of CVD. Bloembergen et al have shown that for each 0.1 higher Kt/V, the adjusted risk of death from CAD was 9% lower, while that due to other causes of cardiac disease was 12% lower. The comparative CVD mortality between patients treated by haemodialysis and peritoneal dialysis remains controversial. Data from the USRDS show a higher risk of death attributable to CAD and other cardiac causes in peritoneal dialysis patients, after adjusting for age, race, gender, cause of ESRD, and time on dialysis. This is in contrast to data from the Canadian Organ Replacement Registry, which shows a lower overall mortality in peritoneal dialysis patients. The type of dialysis membrane may also be important. Some evidence suggests that patients dialyzed with cellulose membranes (in comparison to synthetic membranes) appear to have a higher risk of death attributable to CAD. This observation needs be evaluated further in prospective studies.

1.6.3 NKF Task Force Recommendation

Based on these data and others, the NKF Task Force made the following recommendation regarding the epidemiology of CVD risk factors in CRD. "The excess risk of CVD in CRD is caused, in part, by a higher prevalence of conditions that are-recognized as risk factors for CVD in the general
lotin such as older age, hypertension, hyperlipidaemia, diabetes, and physical inactivity. The excess risk may also be caused, in part, by haemodynamic and metabolic factors characteristic of CRD, including proteinuria, increased extracellular fluid (ECF) volume, electrolyte imbalance, anaemia, and higher levels of thrombogenic factors and homocysteine than in the general population. Strategies for risk factor identification and reduction should target both the traditional coronary risk factors and specific risk factors related to CRD." "Patients with CRD should be considered in the highest risk group for subsequent CVD events. They have a high prevalence of CAD and LVH, which are precursors of CVD mortality and morbidity. They also have a high prevalence of CHF, which is an independent predictor of death in CRD. Treatment recommendations based on CVD risk stratification should consider the 'highest risk' status of patients with CRD.

1.7 PARALLELS BETWEEN CVD AND CRD

1.7.1 Pathogenesis

The similarities between the pathogenesis of atherosclerosis and glomerulosclerosis have been reviewed by various investigators. The histologic features common to both conditions include influx of monocytes, production of lipid-laden macrophages, presence of cholesterol and cholesterol esters, proliferation of contractile cells (either vascular smooth muscle cells or glomerular mesangial cells), and expansion of both
oilagenous and noncollagenous matrix expansion resulting in fibrosis. The striking similarities in the histological appearance of atherosclerosis and glomerulosclerosis suggest that mechanisms for the two processes may be similar.

1.7.2 Risk Factors

Many of the risk factors for development of CVD and CRC are similar. Older age, male gender, diabetes, hypertension, and proteinuria have all clearly been shown to be risk factors for both CVD and CRD. There is some evidence suggesting that other traditional and uraemia-related risk factors for CVD may also hasten the progression of renal disease.

Elevated LDL cholesterol is clearly implicated in the progression of some experimental models renal disease, but there are little data in humans.\(^{[89]}\) However, low HDL and elevated triglyceride levels have been shown to be independent predictors of renal function decline in some studies.\(^{[90]}\)\(^{[91]}\) Lp(a) has been shown to stimulate cell proliferation and DNA synthesis in rat mesangial cells.\(^{[92]}\) Furthermore, Lp(a) has been demonstrated in the glomeruli of patients with glomerular disease.\(^{[92]}\) Therefore, it is reasonable to hypothesize that some atherogenic lipids are risk factors for renal disease progression.

Homocysteine may cause atherosclerosis through enhanced LDL oxidation and smooth muscle proliferation.\(^{[67]}\) Similar mechanisms have been implicated in progressive glomerulosclerosis. Furthermore, a recent study showed that elevated tHcy was an independent risk factor for
 Albuminuria, which is in turn a well-documented risk factor for renal disease progression. 

Many studies have shown a key role for inflammatory mediators in the progression of renal disease. In the MDRD study, a lower transferrin (a negative acute-phase reactant) level was an independent risk factor for renal disease progression.

1.8 LESSONS FROM CVD PREVENTION IN THE GENERAL POPULATION

Strategies for primary prevention of CVD are defined as measures to reduce the risk of developing the first manifestation of CVD. Risk factor reduction strategies include intervention for individuals at risk, such as diet or drug therapy for hypertension and hyperlipidaemia, and public health measures, such as dietary modification or increasing the level of physical activity, to lower the burden of risk across the population. Strategies for secondary prevention of CVD are defined as interventions to reduce subsequent CVD outcomes, such as nonfatal events or mortality, among patients already known to have CVD. These interventions include screening for disease, treatment of specific CVD (specifically CAD and LVH), and risk factor reduction. Clinical practice guidelines and recommendations from national committees, such as the Joint National Committee for the Prevention,
Detection, Evaluation, and Treatment of High Blood Pressure and the National Cholesterol Education Program Adult Treatment Panel, are based on the highest level of evidence available from observational studies and clinical trials. In general, recommendations have been developed for most of the "traditional" CVD risk factors. In most cases, the NKF Task Force recommended applying the guidelines and recommendations developed for reduction of traditional CVD risk factors in the general population to patients with CRD.

Because of the parallels between CVD and CRD, we suggest that it may be useful to adopt some of the same methods to develop guidelines and recommendations for strategies to prevent the progression of CRD.

CRI represents a critical period in the evolution of kidney failure; it is during this period that the most troublesome and perplexing complications of kidney failure begin their insidious courses. As physicians who care for patients on dialysis, we are frustrated with the common occurrence of long-term complications, such as metabolic acidosis, malnutrition, and renal osteodystrophy, as well as the co morbidities of poorly controlled hypertension, advancing diabetes, atherosclerotic vascular disease, and congestive heart failure and other manifestations of left ventricular dysfunction. These are complex and often inter related clinical problems that are difficult to manage, cause significant morbidity and mortality, and generate enormous costs to our health care system.
We must realize that the seeds for these complications are sown very early in the disease course of CRI. They are initially sub clinical, but nonetheless progress relentlessly to what may eventually become a symptomatic and irreversible state. Early in the course of CRI, they are most amenable to intervention with relatively simple therapies that have the potential to prevent very complicated—and adverse-outcomes. These conditions are currently under recognized and available therapies are under utilized. By acknowledging these facts, we have an excellent opportunity to change the paradigm of CRI management and significantly improve patient outcomes.
I JUSTIFICATION OF THE STUDY

Chronic Renal Insufficiency and its complications including cardiovascular problems constitute a major and growing health problem both locally and globally. The increasing population of Diabetics, Hypertensives and the elderly in Kenya serves to indicate a future rise in the population of patients who are at risk of progressing to End Stage Renal Disease. If health care priorities remain as they are now, this tide of disease may overwhelm existing infrastructure. Hence strategies to stem the progression of this population-at-risk must be instituted. Central to this, is the recognition of the disease entity of chronic renal insufficiency and comprehensive management of these patients.

As the major cause of mortality and morbidity in this population is cardiovascular, (1,5) it is essential to identify risk factors in order to effectively manage these patients. It has been suggested by certain schools of thought that this population be treated as a high priority group when it comes to effecting preventive measures against cardiovascular disease.

There is no local data on the prevalence of co-existing cardiovascular risk factors among our chronic renal insufficiency population. There is no available data from within the African continent. Most Data available currently emanates from the developed world and this may not accurately reflect our situation due to major socio-cultural, economic and environmental differences.
This study proposed to determine the prevalence of certain established risk factors of cardiovascular disease in patients with chronic renal insufficiency. The data generated from this study will assist in assessing the burden of specific established cardiovascular risk factors in our chronic renal insufficiency population in order to facilitate the planning and conducting of further detailed studies on cardiovascular morbidity in this population, and in setting up public health intervention programmes for primary and secondary prevention of cardiovascular disease in these patients, this being far more cost effective than the treatment of established CVD, especially in developing nations like ours with extremely limited resources.
3. OBJECTIVES

3.1 BROAD OBJECTIVE

10 determine the prevalence of certain established and emerging cardiovascular risk factors, and to describe possible associations of these risk factors, in black CRI patients attending the Kenyatta National Hospital.

3.2 SPECIFIC OBJECTIVES

A. To determine the prevalence of the following cardiovascular risk factors among black chronic renal insufficiency patients attending the Kenyatta National Hospital.

1. Cigarette smoking
2. Obesity
3. Systemic arterial hypertension
4. Dyslipidaemia
5. Poor glycaemic control in Diabetic patients.
6. Hyperhomocysteinaemia
7. Left ventricular hypertrophy
8. Anaemia

B. To describe the possible clustering of these risk factors among patients with chronic renal insufficiency.

To explore the possible relationship between anaemia, hypertension and left ventricular hypertrophy.
4.1 STUDY DESIGN

Hospital based, cross-sectional descriptive study

4.2 STUDY POPULATION

Patients with chronic renal insufficiency, seen and followed up at Kenyatta National Hospital during the study period.

4.3 STUDY DURATION

Eight months starting April 2002 to October 2002.

4.4 SAMPLING TECHNIQUE

All consecutive patients who satisfied the inclusion criteria during the study period were recruited into the study.
The sample size for this study had been estimated using a statistics table for descriptive studies, which was based on the following formula. The study sample size was designed for a 90% confidence level and a total width of confidence interval of 0.20.

\[ N = 4za^2 \times P \times (1-P) \times W^2 \]

Where \( N \) is the calculated sample size, \( P \) is the expected proportion, \( W \) is the total width of confidence interval and \( za \) is the standard normal deviate for a two-tailed alpha.

The prevalence of each of the risk factors that was to be determined in this study was indicated in the following table, having been established from previous studies.(1),(13)
TABLE 5.

<table>
<thead>
<tr>
<th>Factors for CVD</th>
<th>Prevalence (Other Studies) [1,15]</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of previous CVD</td>
<td>38.50%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>79.90%</td>
</tr>
<tr>
<td>Dyslipidaemias</td>
<td>43.40%</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>37.50%</td>
</tr>
<tr>
<td>Smokers</td>
<td>27.30%</td>
</tr>
<tr>
<td>Hyper-homocysteinaemia</td>
<td>80%</td>
</tr>
<tr>
<td>Left ventricular hypertrophy</td>
<td>30%</td>
</tr>
<tr>
<td>Obesity</td>
<td>30%</td>
</tr>
<tr>
<td>Anaemia</td>
<td>30%</td>
</tr>
</tbody>
</table>

Thus the minimum sample size necessary was 40 patients and the study actually recruited 83 patients.
4.6.1 INCLUSION CRITERIA

1. Patients with a diagnosis of Chronic Renal Insufficiency based on the following definition;

   CRI will be defined as, calculated creatinine clearance (Cockcroft-Gault) less than 75 mL/min (documented twice at least 1 month apart), with no identifiable reversible cause. (1),(2)

2. A duly signed informed consent from the patient.

3. Subjects must be Black.

4.6.2 EXCLUSION CRITERIA

1. Patients who were dialysis dependent or who fulfilled absolute indications for maintenance dialysis, which are generally accepted by nephrologists to be;

   A. Pericarditis

   B. Fluid overload or pulmonary oedema refractory to diuretics

   C. Accelerated hypertension poorly responsive to anti-hypertensive medication.

   D. Progressive uraemic encephalopathy or neuropathy, with signs such as confusion, asterixis, myoclonus, wrist or foot drop, or in severe cases, seizures

   E. A clinically significant bleeding diathesis attributable to uraemia
F Persistent nausea and vomiting

G. Plasma creatinine concentration above 12 mg/dL (1060 pmol/L) or blood urea nitrogen (BUN) greater than 100 mg/dL (36 mmol/L)

2. Pregnant women
3. Patients with or suspected to suffer from conditions which may have interfered with the assay of glycated haemoglobin fraction, such as haemoglobinopathy or lead poisoning.

4. Patients on drugs such as methotrexate, carbamazepine or phenytoin, which may affect plasma homocysteine concentration.

5. Patients with pre-existing valvular heart disease, which may have interfered with interpretation of LVMI values.

6. Any patient who declined to consent to participating in the study.
7 PATIENT EVALUATION

During the study period, files of all patients booked to the medical outpatient clinics were scrutinised, the files were obtained from the medical records officer before starting the clinic each day. The medical records were examined for pertinent demographic and clinical data. Files of patients with any of the exclusion criteria were left out, ultimately remaining with files of patients likely to be eligible for this study. Underlying cause of CRI was recorded from patient records.

For each of the recruited patients the following were done after an informed consent had been obtained: -

4.7.1 CLINICAL METHODS

I) A complete medical history was taken as per the proforma outlined in appendix 1.

II) A complete physical examination including a thorough cardiovascular examination, was undertaken as per the format outlined in appendix 1.

Standing height was measured once to the nearest 0.5cm, without shoes, the back square against the wall tape, eyes looking straight ahead, with a set square resting on the scalp and against the wall.
Weight was measured once with a lever balance to the nearest 100 grams without shoes, in light garments.

Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared, and was categorised as per the WHO criteria [95]. Waist circumference (WC) in centimetres was taken as the narrowest circumference between the lowest rib and the top of the pelvis, measured in the horizontal plane at the end of gentle expiration, with the subject standing. Hip circumference in centimetres was taken as the maximum circumference in the horizontal plane, measured over the buttocks. Waist-Hip circumference ratio (WHR) was calculated as ratio of the former to the latter, WC and WHR were classified as per dietary guidelines for Americans.[96]

Blood pressure was measured as per the WHO recommendation [97], with the patient in a sitting position and using a standard adult cuff and a manual mercury sphygmomanometer, after an initial rest period of 15 minutes. The systolic blood pressure was determined by the first perception of the Korotkoff sound (Phase 1). Diastolic pressure level was determined by the perception of disappearance of fifth Korotkoff sound (Phase 5). Two measurements at 5 minute intervals were taken and the average of these two readings noted. Hypertension was defined as a systolic or diastolic pressure greater than or equal to 140mm Hg and 90mm Hg respectively, or patients on anti-hypertensive treatment.
4.7.2 LABORATORY METHODS

A.BLOOD

Following 10 to 12 hours of overnight fasting, 12ml blood were drawn by venepuncture from each patient for the following investigations:

1. Serum Urea, Creatinine and Electrolyte assays which were performed at the Department of Clinical Chemistry, M.P.Shah Hospital Laboratory, using the enzymatic Kinetic method for urea measurement, the alkaline picrate reaction for creatinine assay, and the ion-selective electrode method for the assay of sodium and potassium, all with the Random Access clinical chemistry analyser, RA 1000 (Technicon Instruments, USA)

Creatinine clearance was derived from the creatinine levels using the Cockcroft-Gault formula which is shown below.[3]

\[
GFR = \frac{(140 - \text{age})(\text{weight in kilogrammes})}{72(\text{serum creatinine in mg/dl})} * 0.85(\text{for females})
\]

2. Lipid profile assays (total cholesterol, HDL-cholesterol and Triglycerides) were performed at the Department of Clinical
plasma total cholesterol level was determined after enzymatic hydrolysis and oxidation by cholesterol esterase and cholesterol oxidase, using the enzymatic colorimetric test [98],

HDL-cholesterol was assayed by solubilising the HDL lipoprotein particles using a detergent containing polyanion, 4-aminoantipyrine and a buffer solution that releases HDL-cholesterol, which would subsequently be determined after enzymatic hydrolysis and oxidation as for total cholesterol [98].

LDL-cholesterol was assayed by solubilising the non-LDL lipoprotein particles by enzymatic hydrolysis and oxidation as for total cholesterol.

The remaining LDL particles were solubilised using N,N-bis(4-sulfobuty)-m-toluidine disodium and a buffer solution, and a chromogenic coupler which would lead to colour formation [98].

Triglycerides were to be determined after enzymatic hydrolysis by lipoprotein lipase to free fatty acids and glycerol. The glycerol would be phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine disphosphate (ADP). Glycerophosphate would be oxidized to dihydroxyacetone phosphate (DAP) by
glycerol phosphate oxidase producing hydrogen peroxide (H202). In a colour reaction catalysed by peroxidase, the H202 reaction with 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) would be used to produce a red colour dye, the absorbance of which would be proportional to the concentration of triglyceride present in the sample [98].

3. Glycated haemoglobin (HbA1c) assay using the Ion Capture Assay (Abbot IMx SYSTEM) method on automated immunoassay analyser (Imx SYSTEM, USA), was performed at MP.Shah Hospital Laboratories.

4. Plasma homocysteine assay was performed at the VI.P.Shah Hospital Laboratories, using the Imx Homocysteine Fluorescence Polarization Immunoassay (FPI) method, on an automated immunoassay analyser (Imx SYSTEM, USA).

5. Haemoglobin levels were determined by coulter counter method at the KNH laboratories.

B. ECHOCARDIOGRAPHY

All patients were subjected to an Echocardiogram-D and M-Mode) performed by a designated cardiologist (Dr. C. Maina). Measurements of LV mass were derived from two-dimensionally guided M-mode echocardiograms. M-mode measurements were made according to conventions established by the American Society of Echocardiography [99], LV mass was derived from the formula described by Devereux and associates [100].
IV mass (grams) = 0.80 \times 1.04 (VSTd + LVIDd) + PWTdf - (LVIDd)^2 + Qfi

where VSTd is ventricular septal thickness at end diastole, LVIDd is LV internal dimension at end diastole, and PWTd is LV posterior wall thickness at end diastole.

With respect to M-mode LV image quality, if right and left septal and endocardial and epicardial LV posterior wall interfaces could be seen along the entire cycle from which measurements were made, studies would be scored as "excellent" (publication quality) or "good" (slightly less optimal edge definition). A score of "fair" implied that at least 5 mm of continuous interface could be seen for each of these four interfaces on contiguous beats but not necessarily on the same beat. A score of "poor" implied that portions of each interface sufficient to make a measurement could be extrapolated from three consecutive cycles. The LV was scored as "un-measurable" if one or more of the four (septal or LV posterior wall) interfaces could not be identified.[99]
4.8 DEFINITIONS OF STUDY VARIABLES

AGE AND SEX

Age and sex as risk factors were defined as >45 years in males and >55 years in females (101).

CIGARETTE SMOKING

Current smokers would have smoked at least 100 cigarettes in their lifetime and are still smoking or would have quit smoking within the preceding year. Former smokers would have smoked at least 100 cigarettes in their lifetime but would have quit smoking more than one year earlier. Subjects who would have smoked less than 100 cigarettes or who had never smoked will be considered never to have smoked. (102)

OBESITY

Waist circumference(cm) was considered abnormal if greater than or equal to 94.0cm in males and greater than or equal to 80.0cm in females.(103,104) Waist-hip ratio was considered abnormal if >0.95 in males and >0.80 in females (103,104). Body mass index (kg/m2) was classified according to the WHO classification (95):

- Normal <25.0
- Overweight 25.0-29.9
- Class 1 obesity 30.0-34.9
- Class 2 obesity 35.0-39.9
- Class 3 obesity >40.0
HYPERTENSION

Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg, or patients on antihypertensives, and was classified as per the WHO classification (97):

Normal <130/85

Borderline 130-139/85-89

Grade 1 140-159/90-99

Grade 2 160-179/100-109

Grade 3 >180/110

DYSLIPIDAEMIA

Dyslipidaemia was classified as per the ATP III (NCEP) guidelines:(105)

Total cholesterol >6.2mmol/L (High)

5.17 - 6.18mmol/L (Borderline High)

<5.17 (Desirable)

LDL - cholesterol <2.58mmol/L (optimal)

2.58 - 3.33 (Near optimal)

3.34 - 4.11 (Borderline high)

4.13 - 4.88 (High)

>4.91 (Very high)

HDL - cholesterol <1.03mmol/L (Low) and >1.55mmol/L (High)

Triglyceride level >2.26 mmol/L (High)
**HOMOCYSTEINE**

Hyperhomocysteinaemia was defined as homocysteine level >10.0 umol/l (68).

**DIABETIC CONTROL**

Glycated haemoglobin was categorized as (106):

- 4.5-6.0 Excellent control
- 6.0-6.9 Good control
- 7.0-8.0 Marginal control
- >8.0 Poor control

**LEFT VENTRICULAR HYPERTROPHY**

Left ventricular mass index was categorized as per the standards established by the Framingham group. In the healthy, adult, Framingham population, the upper limits of normal were 131 g/m² for males and 100 g/m² for females. (107)

**ANAEMIA**

Haemoglobin levels were categorised according to the established normal values used in Kenyatta National Hospital. (Male<13g/dl,Female<12g/dl) (108)
5. DATA MANAGEMENT

All data from the study was entered into questionnaires and transferred to SPSS 10.0 database, and the data was analysed using SPSS 10.0 software. Continuous data were analysed into means and categorical data into percentages, with their corresponding 95% confidence internals. Comparisons of continuous data were made using the t test, and of categorical data using the chi-square test or fishers exact test. Correlations between continuous variables were tested using Pearson correlation coefficient.

Prevalence rates of risk factors were calculated as percentages with 95% confidence intervals. Association of multiple risk factor variables were determined, and correlations between some of these variables were also identified as described above. Clustering (co-occurrence) of risk factors was described as number of risk factors present.

Statistical significance was defined as a two-tailed p value of less than or equal to 0.05.
6. ETHICAL CONSIDERATIONS

The study was conducted after approval by the ethical committee, Kenyatta National Hospital. Cases were only included after going through the consent process. The cases were informed that the project involved research. They were also told of the purpose of the research. The procedures of the study were clearly explained giving full details on all blood and imaging tests. Medical and psychological harms and benefits were described in lay terms. They were also assured that participation in the study was voluntary, and that declining to participate or withdrawal from the study would not result in any penalty or loss of benefits. The cases were assured of full and free access to their results and that all necessary therapeutic interventions would be made according to accepted standards of practice. It was also affirmed that confidentiality would be strictly maintained. All data obtained was securely stored and that there was no cost incurred by the cases (as all costs were carried by the investigator.) Following this, an offer to answer questions or provide further information was made. In the event of a favourable response the patient was requested to sign an informed consent form. (Appendix III)

All costs of the study were borne by the principal investigator from personal sources.
7. RESULTS

A total of 834 patients were screened between the 30th April 2002 and the 10th of October 2002, this comprised; 116 patients with hypertension, 310 with various glomerulonephropathies, 298 with chronic renal failure of varying severity, 64 with recurrent urinary tract infection, and 46 with miscellaneous conditions. Of these, 93 patients satisfied the criteria of CRI, Eighty-three patients met study requirements, while ten patients were excluded. Of the ten excluded patients, four withdrew consent, four patients did not return for follow up, and two patients had significant valvular heart disease. Data for 83 patients was analysed.

DEMOGRAPHICS

There were 59 male patients (71%) and 24 female patients (29%), giving a male to female ratio of 2.5:1. The mean age of the population studied was 52.7 years, with a range of 28 years to 72 years. The number of male patients above 45 yrs of age and female patients older than 55 yrs of age was 52 (62.7%), while those of males and females less than 45yrs and 55yrs respectively was 31 (37.3%).

AETIOLOGY OF CHRONIC RENAL INSUFFICIENCY

The cause of the chronic renal insufficiency, as indicated in patient records, was Chronic glomerulonephritis in 30 patients (36.1%), Hypertension in 26 patients (31.3%), Diabetes in 24 patients (28.9%), and other causes in 3 patients (3.6%).
Fig1: AETIOLOGY OF CRI.

3.6%
28.9%
31.3%
36.1%

DEGREE OF RENAL DYSFUNCTION

Creatinine clearances ranged from 17mls/min to 75 mls/min. The mean creatinine clearance was 47.072 mls/min. Fourteen patients (16.9%) had a creatinine clearance of less than 25mls/min, twenty nine patients (34.9%) had a creatinine clearance between 25mls/min and less than 50mls/min; and forty patients (48.2%) had creatinine clearances of between 50mls/min and 75mls/min.

TABLE 6: Distribution of study population by Creatinine clearance

<table>
<thead>
<tr>
<th>CREAT.CLEARANCE</th>
<th>NO. OF PATIENTS</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25 mls/min</td>
<td>14</td>
<td>16.9%</td>
</tr>
<tr>
<td>25-49mls/min</td>
<td>29</td>
<td>34.9%</td>
</tr>
<tr>
<td>^0-75mls/min</td>
<td>40</td>
<td>48.2%</td>
</tr>
</tbody>
</table>
Cigarette Smoking

The prevalence of current cigarette smoking was 7.23% (six patient?) who averaged 15 pack-years of smoking. Ten patients (12.06%) were ex-smokers. The average 6 pack-year§ of current smokers were while only nrtp of the ex-smokers was a female.

Fig 2: Distribution of study participants according to smoking status

Qbesity

BMI

The mean BMI was 23.4kg/rrr, with a range of 16-31kg/rrf. Fifty six patients (S7 5%) were neither overweight nor obese. Twenty six patients (31 3%) were overweight and one patient (1.2%) had class 1 obesity. 94% of males and of females were overweight/or obese.

twenty seven (32.5%) patients were either overweight or obese.
Central obesity was measured using the WHR/WC. The average WHR was 0.38 and the average WC was 90.4 cm. 13 males (22% of all males) and 18 females (75% of all females) had elevated WHR, while 25% (15) of males and 79% (19) of females had abnormal WC. The average male WHR was 0.91 and the average WC was 91.9 cm, while the average female WHR was 0.82 and the average WC was 86.5 cm. Females were significantly more likely to be centrally obese than males (p=0.033). Fifteen (55%) of the twenty-seven patients who were overweight or obese (by BMI) had central obesity. Of the fifteen patients who had central obesity and an elevated BMI, eight (53.3%) were diabetic, four (26.7%) were hypertensive, and three (20%) had chronic glomerulonephritis as their underlying illness.

**Fig 3: Classification of obesity according to BMI**

<table>
<thead>
<tr>
<th>BMI</th>
<th>Normal</th>
<th>Overweight</th>
<th>Obese 1</th>
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<td>0</td>
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<td>100</td>
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</tbody>
</table>
SYSTEMIC ARTERIAL HYPERTENSION

The mean systolic blood pressure was 140.5 mmHg with a range of 110-170 mmHg. The mean diastolic blood pressure was 88.6 mmHg with a range of 60-115 mmHg. Twenty-three patients (27.7%) were normotensive, nine (10.8%) patients were borderline hypertensives, thirty four patients (41%) had grade 1 hypertension, and seventeen patients (20.5%) had grade 2 hypertension. There were strong co-relations between hypertension and left ventricular mass index (p=0.00003), as well as hypertension and anaemia (p=0.00050). 81% of patients with elevated BP were on anti-hypertensive treatment. 73% of them were on more than one agent, while 27% of them were on a single agent. BP control was uniformly poor.
**Total Cholesterol:**

The mean Total cholesterol level was 3.909 mmol/L (95% CI 2.41-5.4).

Sixty six (79.5%) patients had total cholesterol levels within the desirable range, thirteen patients (15.7%) had levels considered as borderline, while four patients (4.8%) had frankly elevated levels. There was no significant gender difference, nor was there an effect of the underlying aetiology of CRI.
Fig 6: Total cholesterol levels in study population

**LDL Cholesterol:**

Mean LDL-cholesterol level was 2.36mmol/L (95% CI 1.02 -3.69)

Fifty three patients (63.9%) had optimal levels of LDL cholesterol. Ten patients (12%) had near optimal control, ten patients (12%) had borderline high levels, eight patients (9.6%) had high levels, while two patients (2.4%) had very high levels. There was no significant gender difference, nor was there an effect of the underlying aetiology of CRI.
HDL Cholesterol:

Mean HDL Cholesterol level was 0.862 (95% CI 0.474-1.25).

Seventy three patients (88%) had low HDL Cholesterol levels. nine(10.8%) had normal levels, while one patient(1.2%) had high levels. All twenty four diabetic patients had low levels (100% of diabetics), twenty three hypertensives had low levels (88.5% of all hypertensives), while twenty four patients with an underlying diagnosis of chronic glomerulonephritis had low levels (80% of all chronic glomerulonephritis patients), these differences were not statistically significant. There were no significant gender differences.
TRIGLYCERIDES:

Mean Triglyceride level was 1.252 mmol/L (95% CI 0.426-2.076)

Seventy patients (84.3%) had normal levels, while thirteen patients (15.7%) had high levels. Eight of the thirteen patients with high triglyceride levels were diabetic (33.3% of all diabetics), two were hypertensive (7.7% of all hypertensives), while three had an underlying diagnosis of chronic glomerulonephritis (10% of all chronic glomerulonephritis patients). There was no significant gender difference in hypertriglyceridaemia.
HYPERHOMOCYSTEINAEMIA

The mean homocysteine level was 24.139 \( \mu \text{mol/L} \) (95% CI 10.1-38.1) with a range of 3.55->50 umol/L. Six patients (7.2%) had normal homocysteine levels, while seventy seven (92.8%) had hyperhomocysteinaemia. There was no significant gender difference in hyperhomocysteinaemia, nor was there an effect of the underlying aetiology.
POOR GLYCAEMIC CONTROL AMONGST DIABETIC PATIENTS

The mean HbA1c level was 7.433 with a range of 5-9.9. Four patients (16.7%) had excellent control, seven patients (29.2%) had good control, four patients (16.7%) had marginal control, and nine patients (37.5%) had poor control.
ANAEMIA

The mean Haemoglobin level was 11.67g/dl (95% CI 9.1-14.0), with a range of 1.7g/dl. Forty nine patients (59%) were found to be anaemic. Significantly, creatinine clearance was inversely co-related to haemoglobin levels (p=0.01212). Male patients were more likely to be anaemic than female patients (p=0.00001) there was also a significant relationship between level of blood pressure and haemoglobin level (p=0.0050).

pjq 12: Distribution of patients according to haemoglobin level and creatinine clearance

\[ r = 0.443 \text{(significant)} \]
Fig 13: Distribution of haemoglobin levels according to level of systolic blood pressure. ($r = 0.509$ (significant))

Fig 14: Distribution of haemoglobin levels according to level of diastolic blood pressure. ($r = 0.537$ (significant))
VENTRICULAR HYPERTROPHY

mean left ventricular mass index was 133.6g/m$^2$ (95% CI 94-172), with a range 124-240g/m$^2$. 44 patients (53%) were found to have normal left ventricular mass indices, while thirty nine patients (47%) had high left ventricular mass indices.

Males were more likely to have high left ventricular mass indices ($p = .03800$). There was a significant inverse relationship between creatinine clearance and left ventricular mass index ($p = .00047$).

Similarly, there was a significant inverse co-relation of haemoglobin level and left ventricular mass index ($p < .00001$). There was a strong relationship between level of blood pressure and left ventricular mass index ($p = .00003$).

Fig 15: Distribution of patients according to LVMI and creat. clearance.

$r = 0.541$ (significant)
Distribution of patients according to LVMI and haemoglobin level.

\[ r = 0.748 \text{(significant)} \]

On Multiple regression analysis, there was a significant independent relationship between Haemoglobin level and Left ventricular hypertrophy \( (p < 0.0001) \). Similarly, there was an independent relationship of Hypertension and Left ventricular hypertrophy \( (p < 0.0001) \).
CLUSTERING OF RISK FACTORS

The number of cardiovascular risk factors present in individuals in the study was analysed. These were age and sex (age > 45 years for males and > 55 years for females), cigarette smoking, obesity, high total cholesterol levels, LDL cholesterol levels, low HDL cholesterol levels, hyperhomocysteinaemia, systemic arterial hypertension, poor glycaemic control in diabetics, anaemia, and left ventricular hypertrophy. Most patients (94%) had two or more risk factors present, with the majority (66.3%) having three or more risk factors. The mean number of risk factors per patient were 3.542.
Fig 17: Number of CVD risk factors in study population
RISK FACTOR PREVALENCE IN CRI PATIENTS AT KNH

(IN DECREASING ORDER OF PREVALENCE)

Risk factor | prevalence
--- | ---
Tether homocysteinaeiTiia | 92.8%
lnw HDL cholesterol | 88%
tension | 61%
Anaemia | 59%
LVH | 47%
obesiyy | 32%
rette smoking | 19%

Clustering of risk factors
Mean number of risk factors/patient | 3.5 risk factors
2 or more risk factors | 94%
3 or more risk factors | 66%
8. DISCUSSION

Cardiovascular disease remains one of the most important causes of morbidity and mortality in the chronic renal insufficiency population.

The risk of cardiovascular disease in this population is extremely high. The prevalence of coronary artery disease among haemodialysis patients in the United States is approximately 40%, and irrespective of other co-morbidities, patients with CRI have cardiovascular mortality rates that are at least 10 times greater than the general population. (1)

Data from the UDRDS in 1997 showed a high prevalence of cardiovascular disease in incident and prevalent dialysis patients. Using clinical criteria coronary artery disease and chronic heart failure were each present in 40% of an incident dialysis population—though high. This was probably an underestimation of the true prevalence of coronary artery disease, as shown in angiographic studies of asymptomatic diabetic patients. (14)

There is an increasing global awareness of the importance of cardiovascular risk modification in the chronic renal insufficiency population.

This study was the first of its kind that set out to determine the prevalence of cardiovascular risk factors in the chronic renal insufficiency population, as seen at the Kenyatta National Hospital.

There was no local data available, and no studies from the region that looked at the profile of cardiovascular risk factors in the chronic renal insufficiency
population. Additionally, there are no studies in this regard that have actually focused on a black population.

This study recruited a total of 83 patients, with a male to female ratio of 2.5:1. This was a reflection of the sex distribution of the patients attending the medical outpatient clinics with chronic renal insufficiency. This was unlikely to affect interpretation of results as all risk factors were categorised according to gender specific cut-off values that are internationally accepted. This sex distribution is similar to that found in Tonnelli's study in which males made up 62% of the study participants, this could be explained by the fact that CRI is known to be commoner in males(109). The mean age of the study population was 52.7 years, with 52% of patients having age and sex as a vascular risk factor. On the whole, the average age of patients was significantly younger than that seen in European and American studies. The average age in Tonnelli's study was 62 years(109). This could be explained by the fact that most studies in the west were focused on Caucasian populations, and the fact that our population is significantly younger than western populations. It is also well known from the NHANES III study that black patients develop renal disease much earlier than their white counterparts(27).

There were three major aetiologies of renal insufficiency identified in this population; Chronic Glomerulonephritis, Hypertension, and Diabetes Mellitus. The number of patients in each of the three major aetiological groups mentioned above was similar, with no group dominating the other. This distribution also seemed to reflect generally the proportions of recognised
Aetiologies of chronic renal failure in our population, as described by Kayima, where chronic glomerulonephritis makes up 36%, hypertension 23%, diabetes mellitus 23%, and other causes make up 8%. [110] This distribution is also similar to that described by Kausz and Pereira in their study which was centered around Boston in the United States of America, where Diabetes accounted for 28%, Hypertension for 25% and glomerulopathies for 22%. [111]

Cigarette smoking (current and ex-smokers) was noted in 19.3% of patients. Smokers were significantly more likely to be males. This was the least prevalent risk factor determined in this study. This was not significantly different from rates reported amongst Kenyans by Lore [112], who found a prevalence of 18% among medical students, with males being predominant. Objective markers of smoking such as the analysis of breath carbon monoxide and urine cotinine were not measured. Tonnelli in Canada documented the prevalence of smoking in his study to be 27% (109); this could be attributed to the socio-cultural differences in the two populations that are being compared. The relationship of smoking and cardiovascular disease is well established, and since the relative risk of all cause mortality is much higher in smokers as compared to non-smokers, the importance of abstaining from smoking must be emphasised to patients.

About 32.5% of the patients in this study were overweight or obese. There was no significant difference in the prevalence of obesity between the two
genders. There were also no significant differences among the different aetiological classes. 55% of overweight/obese (by BMI) patients had abnormal waist hip ratios. Central obesity was more frequently found among the diabetic patients, this finding is in keeping with other studies done regionally and in the developed countries. Females were also significantly more likely to have central obesity, this finding was similar to that demonstrated by Vaghela in his study on Type II diabetics.

Obesity is well recognised as a modifiable cardiovascular risk factor. Therefore, obese patients should be encouraged to lose weight (through lifestyle modification and/or anti-obesity medication) and increase the level of exercise.

The prevalence of hypertension in this study was 61.5%. This compares well to figures from western studies (50). 81% of the patients with elevated blood pressure were on anti-hypertensive therapy, with the majority being on more than one agent. This demonstrates the fact that most patients were on sub-optimal therapy for their blood pressure control, and that this presents an opportunity to modify an important risk factor. Hypertension predisposes to all of the major cardiovascular disease outcomes, including cardiac failure, stroke, CHD and PVD. Hypertension was significantly related to anaemia. This was probably attributable to the increased severity of renal dysfunction in the more anaemic patients. The modification of this risk factor is within the realm of the physician and since there are far reaching consequences of elevated blood pressure on both acceleration of the renal disease and
increase in cardiovascular morbidity and mortality, the control of blood pressure should be a priority in this population of patients.

Total cholesterol levels were elevated in 4.8% of patients and borderline in 15.7% of patients. There were no significant gender differences. There were no significant differences according to underlying aetiology of the renal insufficiency. There were also no significant differences noted according to levels of creatinine clearance. This finding is in agreement with western studies that also do not demonstrate increased prevalence of high total cholesterol as compared to the general population.(54)

LDL cholesterol was elevated above optimal levels in 24% of patients. There were no significant gender differences. There were no significant differences according to gender, CRI aetiology, or level of renal dysfunction. Landray et al in Birmingham also found that elevation of LDL was not a significant problem in their population(13). The relatively low prevalence of elevated LDL cholesterol mirrors most western studies where it has been noted that LDL cholesterol levels are not significantly different from those found in the general population (54).

HDL cholesterol was low in 88% of patients. This was the second most prevalent risk factor in this study. There were no significant gender differences. There were no differences according to CRI aetiology and there were no differences noted according to level of renal dysfunction. This is
consistent with studies in Europe, such as Landray's study (13), where it is recognised that HDL levels in renal patients are lower than those found in the general population.(54) This is attributed to the lower activity of lecithin cholesterol acetyl transferase in renal disease.

Triglyceride levels were high in 15.3% of patients. There were no significant sex differences. Diabetics, as expected, showed a trend of having higher triglyceride levels than other groups, but this difference was not statistically significant. There were no significant differences according to levels of renal dysfunction or according to gender.

In Animal models there is a link between dyslipidaemia and progression of renal disease (89). This would provide the clinician with an opportunity to improve both cardiovascular risk and slow progression of renal disease with one intervention. The use of targeted therapies may improve both CVD risk profiles, as well as slow progression of renal disease.

This study revealed an extremely high prevalence of patients with hyperhomocysteinaemia. 92.8% of patients were noted to have hyperhomocysteinaemia, with a mean level of 24.1 •mol/L. This was the most prevalent cardiovascular risk factor noted in this study. There were no differences noted according to sex, CRI aetiology, or levels of creatinine clearance. In western studies prevalence of 90% in patients with ESRD have been found (67). There is now convincing data that mild to moderate hyperhomocysteinaemia is an independent risk factor for CVD in the population(69) Attempts at lowering the homocysteine level can be undertaken
simply by supplementing Vitamin B6, Vitamin B12 and folic acid. This gives the physician a cheaply available opportunity to modify an important risk factor.

Amongst the diabetic patients, the mean level of glycated haemoglobin was 7.3%. This value was similar to that documented by Hsu et al. who found a mean glycated haemoglobin level of 7.5% in the CRI population seen at the Massachusetts General Hospital (114). About half of all diabetics had marginal or poor control of their diabetes. There was no significant gender difference, nor was there any difference according to level of creatinine clearance. The results here indicate the opportunity to improve on glycaemic control in the population studied. This would also provide an opportunity to lower CVD risk, as well as slow the progression of renal dysfunction.

The use of one glycated haemoglobin level may not reflect the long term glycaemic status of these patients, and this was recognised as a limitation of the study.

The mean haemoglobin level was 11.6g/dl. Of all the patients, 59% were found to have anaemia. Males were significantly more likely to be anaemic, this could be attributed to the fact that the degree of renal failure among males in this study was more severe. There was a significant relationship between
anaemia and severity of renal insufficiency, with lower haemoglobin levels being found with more severe renal insufficiency. There was also significant relationship between anaemia and level of hypertension, with higher blood pressure levels being associated with lower haemoglobin levels. This may be attributed to the fact that higher blood pressure levels were found in patients with more severe renal dysfunction. Anaemia is an important remediable risk factor; the use of recombinant human erythropoietin and iron supplementation would translate to improvement in quality of life measures as well as possible reduction in CVD mortality. A study done by Hayashi et al has demonstrated that regression of LVH is possible with the normalisation of Haemoglobin levels in CRI patients (25).

Left ventricular mass index was high in 47% of patients. This is comparable to levels found in other populations around the world. Males were more significantly likely to have higher left ventricular mass indices than females, a fact that could be attributed to the more severe level of renal dysfunction in males in this study. There was no significant difference in LVMI among the various disease groups. There was a significant relation between LVMI and level of creatinine clearance, with LVMI being higher with increase in severity of renal insufficiency. The left ventricular mass index was increased in 85.7% of patients with creatinine clearances of less than 25mls/min, 55.2% of patients with creatinine clearance between 25 and 50mls/min, and 27.5% of patients with creatinine clearances between 50 and 75mls/min. There was also a significant relationship between Haemoglobin level and LVMI, with lower haemoglobin levels being associated with increase in left ventricular
mass index. Left ventricular hypertrophy is an important precursor of cardiovascular morbidity and mortality, and it has been postulated in Levin's study that there is a significant relationship between LVH and anaemia, a result that was mirrored in this study. (10) Levin et al also showed that the prevalence of LVH was inversely related to level of renal function, a finding that was similar to the results in this study. (10)

There was significant clustering of risk factors with 94% of patients having two or more risk factors.

The finding of a high prevalence of various established risk factors in the chronic renal insufficiency population at Kenyatta National Hospital reflects a major opportunity for modification of risk factor profile in these patients, which would result in cost benefits, as well as improvements in morbidity and mortality patterns in this population. Further work is required to quantify risk from the various known factors and evaluate any novel risk factors, as well as assess the burden of clinically silent cardiovascular disease in these patients. Additionally, studies that are interventional in design with a cost-benefit inclination, would be necessary in order to assist selection of an optimal preventive programme for patients with chronic renal insufficiency.
9. CONCLUSIONS

1. There was high prevalence of cardiovascular risk factors present in patients with chronic renal insufficiency seen at KNH.

2. A high proportion of patients had aggregation of multiple risk factors. Most prevalent risk factors were hyperhomocysteinaemia and low HDL cholesterol.

3. Hypertension was prevalent in 61% of the study patients.

4. Glycaemic control was poor in almost half of all diabetics in the study.

5. Hyperhomocysteinaemia was a highly prevalent risk factor.

6. Anaemia was found in 59% of patients and was related to level of renal dysfunction and hypertension.

7. Left ventricular hypertrophy was present in a significant number of study patients and was related to anaemia and level of renal dysfunction.
10. LIMITATIONS

1. Some of the information used in the study required patient recall, which may have been inaccurate.

2. A single HBA1C measurement was used to assess glycaemic control, and this may not reflect long term glycaemic control over many years of diabetes.

3. A search for thrombogenic factors such as increased procoagulant activity and platelet dysfunction could not be undertaken.

4. A set of two blood pressure readings could not be used as a substitute for monitoring of long-term control.
11. RECOMMENDATIONS

1. Cardiovascular risk factors are prevalent and require urgent attention in patients with chronic renal insufficiency.

2. The chronic renal insufficiency population be treated as a high priority group when it comes to effecting preventive measures against cardiovascular disease.

3. More prospective studies are required to identify specific risk factors and their relative risks, using larger samples with case-control or cohort designs, more aggressive diagnosis of vascular disease, involving accurate screening and diagnostic techniques.
12. REFERENCES


12. Parfrey PS, Harnett JD, Foley RN: Heart failure and ischemic heart
disease in chronic uremia. Curr Opin Nephrol Hypertens 4:105-

of known and suspected cardiovascular risk factors in chronic renal

14. US Renal Data System: Comorbid conditions and correlations with
mortality risk among 3,399 incident hemodialysis patients. Am J Kidney Dis
20:S32-S38, 1992 (suppl 2)

National Institutes of Health, National Institute of Diabetes and Digestive and
Kidney Disease, 1998

Canadian Organ Replacement Register, Canadian Institute for Health
Information, Ottawa, Canada, 1998

South Australia, Australia and New Zealand Dialysis and Transplant Registry,
1996

18. Hunter JJ, Chien KR*: Signaling pathways for cardiac hypertrophy and


64. Cressman MD, Heyka RJ, Paganini EP, O'Neil J, Skibinski CI, Hoff HF: Lipoprotein(a) is an independent risk factor for cardiovascular disease in hemodialysis patients. Circulation 86:475-482, 1992


97. World Health Organization-International society for
hypertension. J. Hypertension. 1999; 17:151-83


pneumococcal disease. NEJM 2000;342:681-9


108. Kenyatta National hospital Laboratory Normal values.


13. APPENDICES

13.1 APPENDIX I STUDY PROFORMA

STUDY NO.

NAME

DATE HOSPITAL NO.

AGE (Years) DOB (M/Y)

DATE OF DIAGNOSIS OF RENAL DYSFUNCTION

PRESUMED AETIOLOGY OF RENAL DYSFUNCTION

DEMOGRAPHICS

1. GENDER

MALE
NEVER HAD FORMAL EMPLOYMENT

RETIRED

5. LEVEL OF FORMAL EDUCATION

NONE

PRIMARY SCHOOL

SECONDARY SCHOOL

TERTIARY

OTHER(specify)

PAST MEDICAL HISTORY

HAVE YOU EVER HAD ANY OF THE FOLLOWING?

"Y" for yes and "N" for No
BEN TOLD BY A DOCTOR YOU HAD CORONARY HEART DISEASE

HEART ATTACK

ANGINA PECTORIS (CHEST PAIN DUE TO REDUCED BLOOD FLOW TO HEART)

CORONARY BYPASS SURGERY

CORONARY ANGIOPLASTY (BALOONING)

ABDOMINAL AORTIC ANEURYSM

BLOCKAGE OF LIMB ARTERIES

TRANSIENT ISCHEMIC ATTACKS (MINI STROKE)
BLOCKAGE OF CAROTID ARTERY

STROKE

FAMILY HISTORY

DID OR DO ANY OF YOUR RELATIVES SUFFER FROM

1. DIABETES
   YES  NO

(If Yes, specify and give relation to yourself)

Relationship:  Father
   Mother
   Brother/sister
   Children
   Other (specify)

2. HYPERTENSION
   YES  NO

%  

Relationship:  Father
   Mother
Brother/sister
Children
Other (specify)

KIDNEY DISEASE  YES

Relationship:  Father
Mother
Brother/sister
Children
Other (specify)

HEART ATTACK  YES

Relationship:  Father
Mother
Brother/sister
Children
Other (specify)

STROKE  YES
Relationship: Father
Mother
Brother/sister
Children
Other (specify)

6. SUDDEN DEATH YES NO

Relationship: Father
Mother
Brother/sister
Children
Other (specify)

SMOKING HABITS

1. Are you currently smoking cigarettes?

Yes

No

a) If "yes" how many do you smoke per day?

cigarettes/day
b) How many did you smoke per day a year ago?
cigarettes/day
c) How long have you been smoking? Years

2. Did you ever smoke cigarettes?

Yes (state when you gave up)

No

a) If "yes" what is the maximum number you ever smoked per day (for at least a year)? cigarettes/day

3. Do you drink alcohol?

Yes

No

If "yes" quantify Units per day
CURRENT MEDICATION:

Are you currently on any of the following medications;?

1. DRUGS TO LOWER BLOOD SUGAR (ORAL/INJECTABLE)

Yes

No

If "yes" specify

Drug

Dose

Duration
2. BLOOD PRESSURE LOWERING DRUGS

Yes

No

If "yes" specify

Drug

Dose

Duration

3. BLOOD LIPID LOWERING DRUGS

Yes

No

If "yes" specify

Drug
4. ANY OTHER DRUG TAKEN REGULARLY, AT LEAST ONCE/DAY

Yes

No

If "yes" specify

Drug

Dose

Duration

PHYSICAL EXAMINATION

HEIGHT (cm)
WEIGHT (kg)

BMI (kg/m²)

WAIST CIRCUMFERENCE (cm)

HIP CIRCUMFERENCE (cm)

WHR

BLOOD PRESSURE (mmHg)
(Average of 2 readings)

EYES

1. ARCUS SENILIS
   Yes
   No

2. XANTHELAŞMA
   Yes
NECK

1. RAISED JUGULAR VENOUS PRESSURE

   Yes

   No

2. CAROTID BRUIT

   Yes

   No

HEART

1. APICAL IMPULSE
THRILLS

Yes Specify

No

2. RYTHM  Apical rate /min

Regular

Irregular

Gallop

Other (specify)

3. HEART TONES

Normal

Yes
4. SIGNIFICANT MURMURS

SYSTOLIC

Yes

No

Specify

DIASTOLIC

Yes

No

Specify
13.2 APPENDIX II

LAB RESULTS

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Haemoglobin</td>
<td>g/dl</td>
</tr>
<tr>
<td>Serum Urea</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>mimol/l</td>
</tr>
<tr>
<td>Glycated Haemoglobin (if patient Diabetic)</td>
<td>%</td>
</tr>
<tr>
<td>Homocysteine level</td>
<td>mimol/l</td>
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Serum Lipid profile

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Total Cholesterol</td>
<td>mmol/l</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>mmol/l</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>mmol/l</td>
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</table>

Echocardiography findings

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>LeftA/entricular mass index</td>
<td>grammes/m²</td>
</tr>
</tbody>
</table>
CONSENT FORM

I, ............................................................................................................................................. Do
voluntarily agree to take part in this research study on CARDIOVASCULAR
RISK FACTORS IN CHRONIC RENAL INSUFFICIENCY IN BLACKS as
seen at the Kenyatta National Hospital. The nature and purpose of the study
has been explained to me, additionally I am clearly aware of the procedures
required (drawing of blood and Echocardiography). I also clearly understand
the possible risks and benefits involved and that my participation is purely
voluntary. DR.NADEEM SHEIKH has given all of the above information to me.

SIGNED

WITNESSED

DATED