THE ROLE OF CIRCULATING ENDOTOXAEMIA AS A PROINFLAMMATORY MEDIATOR OF ATHEROSCLEROSIS IN CHRONIC KIDNEY DISEASE PATIENTS

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of

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DECLARATION

I, Muzamil Olamide Hassan hereby declare that this thesis is original work done by me. It is being submitted for the degree of Doctor of Philosophy (Internal Medicine) in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted previously for any degree or examination at this or any other University.

22nd of June, 2015.

DEDICATION

I dedicate this work:

To my loving and supporting wife Mutiyat Adeyoola, my children, Sultan and Ahmad for bearing with my long absence from home

To my parents, Alhaji Yunus Hassan and Alhaja Maryam Hassan for supporting me throughout this journey

To almighty Allah, the most gracious and the most merciful

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

A. Manuscript submitted for Publication

1. Assessment of volume overload and its risk factors in South African chronic kidney disease patients: an appraisal of bio-impedance spectroscopy and inferior vena cava diameter measurements – submitted to Nephrology, Dialysis and Transplantation (Appendix1)

B. Abstracts

1. Hassan M, Duarte R, Dix-Peek T, Vachiat A, Grinter S, Naidoo S, Manga P, Naicker S. Endotoxaemia: An independent risk factor for atherosclerosis in chronic kidney disease patients. (Abstract CS/TH-O-14). Wits Faculty of Health Science Research Day and Postgraduate Expo 2014 Abstract Book 2014 - published on line: South African Journal of Infectious Diseases, 2015; 30 (1): 4.
2. Hassan M, Duarte R, Dix-Peek T, Vachiat A, Grinter S, Naidoo S, Manga P, Naicker S. Immune activation contributes to severity of endotoxaemia related atherosclerosis in chronic kidney disease patients. World Congress of Nephrology, March 13th – 17th, 2015. Cape Town, South Africa. Abstract WCN15-1010, page 145, WCN 2015 Congress Program Book.
3. Hassan M, Duarte R, Dix-Peek T, Vachiat A, Grinter S, Naidoo S, Manga P, Naicker S. Correlation between volume overload, systemic inflammation and left ventricular dysfunction in chronic kidney disease patients: an appraisal of methods of hydration status assessment. World Congress of Nephrology, March 13th – 17th, 2015. Cape Town, South Africa. (Abstract WCN15-1012), page 345, WCN 2015 Congress Program Book.
4. Hassan M, Duarte R, Dix-Peek T, Vachiat A, Grinter S, Naidoo S, Manga P, Naicker S. Oxidized low density lipoprotein is an independent risk factor for cardiovascular disease in chronic kidney disease patients. World Congress of Nephrology, March 13th – 17th,

2015. Cape Town, South Africa. (Abstract WCN15-1013), page 345, WCN 2015 Congress Program Book.

5. Hassan M, Duarte R, Dix-Peek T, Naidoo S, Naicker S. The relationship between renal function, volume overload and circulating endotoxaemia in CKD patients. (Abstract NAN/CN/2015 – 10). In: Book of Abstracts, 27th Nigeria Association of Nephrology Congress, January 25th – 29th 2015, Calabar, Nigeria, p.35.
6. Hassan M, Duarte R, Dix-Peek T, Vachiat A, Grinter S, Naidoo S, Manga P, Naicker S. Circulating endotoxin is related to systemic inflammation and atherosclerosis in South African CKD patients. (Abstract NAN/CN/2015 – 12). In: Book of Abstracts, 27th Nigeria Association of Nephrology Congress, January 25th – 29th 2015, Calabar, Nigeria, p.37.

ABSTRACT

Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Moreover, endotoxin release into the circulation is a potential source of inflammation, and represents an important target for intervention directed towards stemming of cardiovascular morbidity and mortality in chronic kidney disease (CKD) patients. Although previous studies have shown that elevated plasma endotoxin levels were associated with the risk of atherosclerosis, a direct link between endotoxaemia and atherosclerosis is yet to be fully elucidated. This study investigated endotoxaemia across the spectrum of South African CKD patients and also determined the pro-inflammatory effects of endotoxin that are relevant to the development of subclinical atherosclerosis in CKD patients.

A prospective cross-sectional study of 120 CKD patients was carried out, comprising 40 each of peritoneal dialysis (PD), haemodialysis (HD) and stage 3 chronic kidney disease (CKD), attending the Charlotte Maxeke Johannesburg Academic Hospital, South Africa; and a comparator cohort of 40 age- and sex-matched controls. Endotoxin levels were measured and the results compared with serum lipopolysaccharide binding protein (LBP), serum CD14 (sCD14), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), oxidized LDL, interleukin-6 (IL-6), C-reactive protein (CRP), transforming growth factor-β (TGF-β), carotid intima media thickness (CIMT) and plaque occurrence (measured by B-mode ultrasound), and echocardiographic parameters. Bio-impedance spectroscopy was carried out using a body composition monitor (BCM) to assess fluid and nutritional status. Inflammatory cytokines including TGF-β1 and IL-6 were genotyped, and the genotype frequencies compared to the serum concentrations of the cytokines. Data was analysed using the statistical package for social sciences (SPSS) version 16.

Volume overload and circulating endotoxaemia were common across the spectrum of CKD patients, and were aggravated by worsening kidney function. Endotoxins levels correlated

with both absolute overhydration (r=0.513, p<0.001) and percentage overhydration to extracellular water (OH/ECW%) (r=0.490, p<0.001). Carotid intima media thickness was significant higher among CKD patients compared to the controls (0.58 ± 0.12 versus 0.45 ± 0.05 mm; p<0.001); CIMT was 0.63 ± 0.12; 0.56 ± 0.09 and 0.56 ± 0.12 mm in PD, HD and CKD stage 3 patients respectively. Carotid intima media thickness correlated with overhydration (r=0.442, p<0.001) and OH/ECW% (r=0.421, p<0.001). The risk of atherosclerosis was associated with serum levels of endotoxins (odds ratio: 2.34; confidence interval: 1.26-4.35, p=0.007), with excess risk confined to the group with high endotoxin levels. The risk of subclinical atherosclerosis was predicted by high concentrations of sCD14, IL-8 or MCP-1. Patients with high levels of circulating endotoxaemia in combination with high levels of sCD14, IL-8 or MCP-1 showed markedly elevated risk of atherosclerosis. The lowest Oxidized LDL levels were significantly higher in patients with carotid plaques compared to patients without carotid plaques (472.6 ± 104.6 versus 348.6 ± 110.9 ng/ml, p=0.012). Oxidized LDL showed a strong association with CIMT (r=0.664, p<0.001) among non-dialytic CKD patients. TGF-β isoform were present in lower concentrations in haemodialysis patients compared to the PD, CKD and controls. TGF-β concentrations were significantly lower in the patients with subclinical atherosclerosis compared to patients without atherosclerosis (TGF-β1: $4.1 \times 10^4 \pm 1.5 \times 10^4$ versus $5.9 \times 10^4 \pm 1.6 \times 10^4$ pg/ml, p<0.001; TGF-β2: $1.6 \times 10^3 \pm 0.3 \times 10^3$ versus $1.8 \times 10^3 \pm 0.2 \times 10^3$ pg/ml, p<0.001; and TGF-β3: $4.2 \times 10^2 \pm 0.9 \times 10^2$ versus $5.3 \times 10^2 \pm 1.1 \times 10^2$ pg/ml, p<0.001). TGF-β isoforms had an inverse relationship with CIMT (TGF-β1: r=−0.562, p<0.001; TGF-β2: r=−0.477, p<0.001; TGF-β3: r=−0.442, p<0.001). There was significant association between IL6 polymorphisms (−174G/G and −174G/C) and elevated serum IL-6 levels in CKD patients.

Volume overload and circulating endotoxaemia were common across the spectrum of CKD patients. Volume overload was associated with the severity of kidney failure,

inflammation, malnutrition, atherosclerosis and cardiac enlargement. Bio-impedance spectroscopy device is a more sensitive tool than IVCD measurement for the assessment of fluid status in clinically euvolaemic non-dialytic stage 3 CKD patients. The findings suggest that the atherogenic predictive power of endotoxin is significantly increased by the presence of high concentrations of immune mediators in CKD patients. The increased risk of atherosclerosis in CKD patients was demonstrated to be due to the inflammation mediated by endotoxin, with protection afforded by TGB-β. Furthermore, there is a close association between IL-6 polymorphisms (G/G and G/C) and elevated serum IL-6 levels in CKD patients.

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ACEI	angiotensin converting enzyme inhibitor
ADMA	asymmetric dimethylarginine
ADPN	adiponectin
AGES	advanced glycation end products
ADPKD	adult polycystic kidney disease
Apo	apolipoprotein
apo(a)	apolipoprotein (a)
ARBs	angiotensin II receptor blockers
ARIC	atherosclerosis risk in Communities
ASCVD	atherosclerotic cardiovascular disease
BCM	body composition monitor
BMI	body mass index
BIS	bio-impedance spectroscopy
BP	blood pressure
BSA	body surface area
CABG	coronary artery by-pass graft
CAD	coronary artery disease
CHF	congestive heart failure
CIMT	carotid intima-media thickness
CKD	chronic kidney disease
CI	collapsibility index
CP	chronic pyelonephritis /reflux nephropathy
CRF	chronic renal failure
CRP	C-reactive protein

CVD	cardiovascular disease
DBP	diastolic blood pressure
DNA	deoxyribonucleic acid
Echo	echocardiography
ESRD	end-stage renal disease
FGF-23	fibroblast growth factor-23
GFR	glomerular filtration rate
GN	glomerulonephritis
GNB	gram negative bacilli
Hb	haemoglobin
HD	haemodialysis
HDL	high density lipoprotein
HMG-CoA	hydroxy methylglutaryl coenzyme A
Hs-CRP	high sensitivity C-reactive protein
HTN	hypertension
ICAM	soluble intercellular adhesion molecule 1
IDL	intermediate density lipoprotein
IHD	ischaemic heart disease
IL-2	interleukin-2
IL-6	interleukin-6
IL-8	interleukin-8
IMT	intima-media thickness
INF-γ	interferon-γ
IVCD	inferior vena cava diameter
IVSTd	interventricular wall thickness during diastole

KDIGO	Kidney Disease Improving Global Outcomes
LCAT	lecithin- cholesterol acyl-transferase
LDL	low density lipoprotein
LMW	low molecular weight
Lp	lipoprotein
Lp(a)	lipoprotein (a)
LPL	lipoprotein lipase
LPS	lipopolysaccharide
LBP	lipopolysaccharide binding protein
LVEDD	left ventricular internal dimension measurements in end-diastole
LVESD	left ventricular internal dimension measurements in end-systole
LVH	left ventricular hypertrophy
LVM	left ventricular mass
LVMI	left ventricular mass index
MABP	mean arterial blood pressure
MCP-I	monocyte chemoattractant protein-1
MI	myocardial infarction
NA	not available.
NHANES	National Health and Nutrition Examination Survey
NHLS	National Health Laboratory
NKF	National Kidney Foundation
NOS	nitric oxide synthase
NYHA	New York Heart Association
O2−	superoxide anion
oxLDL	oxidized low density lipoprotein

PD	peritoneal dialysis
PTH	parathyroid hormone
PWTd	posterior wall thickness during diastole
ROS	reactive oxygen species
RRT	renal replacement therapy
RWT	relative wall thickness
SBP	systolic blood pressure
SDMA `	symmetric dimethylarginine
SMC	smooth muscle cell
TG	triglyceride
TNF-α	tumour necrosis factor-α
TGF-β	transforming growth factor
TLR-4	toll-like receptor-4
USA	United States of America
USRDS	United States Renal Data System
VCAM-1	vascular adhesion molecule 1
VLDL	very- low- density- lipoprotein