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EMERGING RISK FACTORS AND OUTCOME PREDICTORS OF CORONARY ARTERY DISEASE IN A SRI LANKAN POPULATION

BY

PORUTHOTAGE PRADEEP RASIKA PERERA

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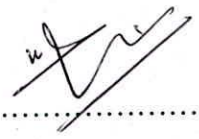
Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Doctor of Philosophy in Biochemistry on 30th January 2009.

DECLARATION BY THE CANDIDATE

The work in this thesis was carried out by me under the supervision of Professor Hemantha Peiris (Head of the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura), Professor Lal Chandrasena (Professor of Biochemistry, Department of Biochemistry, Faculty of Medicine, University of Kelaniya) and Dr. J. Indrakumar (Senior Lecturer, Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura) and a report on this has not been submitted in whole or in part to any University or any other institution for another Degree/Diploma.



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Poruthotage Pradeep Rasika Perera

30.01.2009

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Date

DECLARATION BY THE SUPERVISORS

We certify that the above statement by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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Professor Hemantha Peiris

(Supervisor)

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Professor L. G. Chandrasena

(Supervisor)

.....

Dr. J. Indrakumar

(Supervisor)

Date: 30th January 2009

I dedicate this thesis to

my parents, my wife Chandana

and my daughters

Pavithri, Kaveetha and Dilini.

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ABBREVIATIONS

Apo-A	Apolipoprotein A
Apo B	Apolipoprotein B
BMI	Body Mass Index
CABG	Coronary artery bypasses grafting
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CPK	Creatine phospho kinase
CV	Coefficient of variation
CVD	Coronary Vascular Disease
dATP	deoxy Adenosine Triphosphate
dCTP	deoxy Cytosine Triphosphate
dGTP	deoxy Guanosine Triphosphate
DMD	Duchene's Muscular Dystrophy
DNA	Deoxy ribonucleic acid
dTTP	deoxy Thymidine Triphosphate
EDTA	Ethylenediaminetetraacetic acid
FPIA	Fluorescence Polarization Immunoassay
GNMT	Glycine N-methyltransferase
GPX	Glutathione Peroxidase 1
Hb	Haemoglobin
HDL	High-density lipoprotein cholesterol
HPLC	High Performance Liquid Chromatography
ICD	International Classification of Diseases

IDL	Intermediate-density lipoprotein
IHD	Ischaemic Heart Disease
LCAT	Lecithin cholesterol acyl transferase
LDL	Low-density lipoprotein
Lp(a)	Lipoprotein (a)
MI	Myocardial infarction
MTHFR	Methylenetetrahydrofolate reductase
n	Number
NEB	New England Biolabs
OR	Odds ratio
PAR	Population attributable risks
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PLP	Pyridoxal-5'-phosphate
RBC	Red blood cell
RFLP	Restriction Fragment Length Polymorphism
RNAse	Ribonuclease
ROS	Reactive Oxygen Species
RP-HPLC	Reversed phase HPLC
RV	Reaction vessel
SAH	S-adenosylhomocysteine
SAM	S-adenosyl methionine
SD	Standard deviation
sd LDL	Small dense LDL

TAE	Tris acetate EDTA
TBP	Tri-butyl phosphine
TC	Total cholesterol
TCEP	*Tris*(2-carboxyethyl)phosphine
TG	Triglyceride
SBD-F	7-Fluorobenzofurazan-4-sulfonic acid Ammonium salt
U.K.	United Kingdom
U.S.A.	United States of America
UV	ultra violet
VLDL-C	Very Low-density lipoprotein cholesterol
WHO	World Heath Organization

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Emerging risk factors and outcome predictors of coronary artery disease (CAD) in a Sri Lankan population

PORUTHOTAGE PRADEEP RASIKA PERERA

ABSTRACT

Introduction: Coronary Artery Disease (CAD) is the number one killer disease in Sri Lanka. Apart from the conventional risk factors for CAD, a few new risk factors have been identified. Of these fasting hyperhomocysteinaemia (HHcy), elevated apolipoprotein B, decreased apolipoprotein A-I and the deficiency of the cardiovascular associated antioxidant Glutathione Peroxidase (GPx) have been reported to be significantly associated with CAD.

Objectives: This study was designed to determine a). the association between HHcy and CAD and whether this association was age dependent; b). the likely causes of variations in homocystien levels [mainly changes in serum vitamin B_{12} and folate status, and methylenetetrahydrofolate reductase (MTHFR) A1298C and C677T gene polymorphisms], and c). the association of novel risk factors such as folate, vitamin B_{12}, apolipoprotein A-1, apolipoprotein B, GPx and HHcy in relation to severity of Coronary Artery Disease (CAD).

Methods: A case control study was conducted using 221 subjects with diagnosed acute coronary syndromes and 221 age and sex matched controls to assess the association between HHcy and CAD. The associations between severity of CAD and the other risk factors and folate and vitamin B_{12} with homocysteine were also assessed in 79 subjects with diagnosed CAD.

Results: Results revealed that there was a significant association ($p = 0.002$) between HHcy and CAD. Furthermore, a significant association ($p=0.02$) was observed between

HHcy and CAD in young patients but not in subjects over 50 years of age. HHcy was found to be a significant predictor of CAD after controlling for hypertension and hypercholesterolaemia (adjusted odds ratio 2.411). The vitamin B_{12} and folate levels showed a significantly ($p < 0.01$) negative correlation with serum homocysteine concentrations. There was no significant association ($p>0.05$) between MTHFR C677T and A1298C polymorphisms and homocysteine levels.

Serum homocysteine and folate levels were not significantly related to the severity of coronary artery disease. However, the serum vitamin B_{12} concentrations showed a significant negative correlation with severity of ischaemia when assessed by the vessel score ($p <0.05$) and the extent score ($p < 0.01$). Apolipoprotein A-1 (inversely) and apolipoprotein B/A-1 ratio showed a significant correlation ($p<0.01$) with the stenosis and extent scores but not with the vessel score whilst the apolipoprotein B levels correlated significantly only with the vessel score ($p<0.05$). GPx showed a significant inverse correlation ($p<0.001$) with the vessel, the stenosis and the extent scores. This has not been reported in the literature before.

Conclusions: Hyperhomocysteinaemia is an independent risk factor for CAD and its association is more in the young compared to elderly subjects. While MTHFR gene polymorphisms were not associated with homocysteine concentrations, a decrease in serum concentrations of either vitamin B_{12} or folate was associated with higher homocysteine concentrations. GPx, Apolipoprotein B/A-1 ratio and Apolipoprotein A-1 are better predictors of severity of CAD than apolipoprotein B and homocysteine and they may have a value in assessing the severity of CAD in the future.