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# NOVEL CARDIOVASCULAR RISK MARKERS PARAOXONASE, APOLIPOPROTEIN A-1 (Apo A-I) AND GLUTATHIONE PEROXIDASE GENOTYPE-1 IN CORONARY ARTERY DISEASE

By

WICKRAMASINGHEGE DINUSHKA WICKRAMASINGHE

M.Phil 2014

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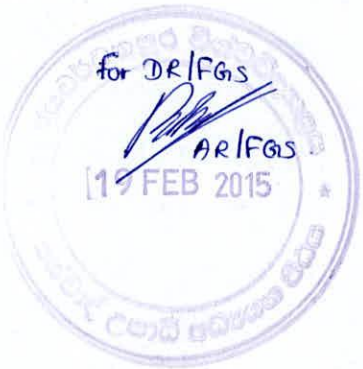


Thesis submitted to the University of Sri Jayewardenepura for the Degree of Master of Philosophy in Biochemistry on 18th August 2014.

## DECLARATION BY THE CANDIDATE

The work in this thesis was carried out by me under the supervision of Professor Hemantha Peiris (Professor of Biochemistry, Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura), Professor Lal Chandrasena (Emeritus Professor, Department of Biochemistry, University of Kelaniya; Director, Clinical Laboratory, Nawaloka Hospitals PLC, Colombo), Dr. Vajira Senarathne (Consultant Cardiologist, Cardiology Unit, National Hospital, Colombo) and Dr. P. P. Rasika Perera (Senior Lecturer, Department of Biochemistry, 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.

.....

Wickramasinghege Dinushka Wickramasinghe

19.02.2015

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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**Dedication**

I dedicate this thesis to

my wife Sugandika, parents and teachers.

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**ABBREVIATIONS**

| | | |
|---|---|---|
| ABCA1 | - | ATP Binding cassette A1 |
| ABCG1 | - | ATP Binding cassette G1 |
| AMI | - | Acute Myocardial Infarction |
| ANOVA | - | Analysis of Variance |
| Apo A-1 | - | Apolipoprotein A-1 |
| Apo J | - | Apolipoprotein J |
| BMI | - | Body Mass Index |
| BP | - | Blood Pressure |
| CABG | - | Coronary Artery Bypass Grafting |
| CAD | - | Coronary Artery Disease |
| CRP | - | C- Reactive protein |
| Cys | - | Cysteine |
| DBP | - | Diastolic blood pressure |
| DM | - | Diabetes mellitus |
| dNTP | - | di Nucleotide Tri Phosphate |
| GI | - | Gastro Intestinal |
| Glu | - | Glucose |
| Gly | - | Glysine |
| GPX | - | Glutathione Peroxidase |
| GR | - | Glutathione Reductase |
| GSH | - | reduced glutathione |

| | | |
|---|---|---|
| GSSG | - | Oxidized glutathione |
| HDL-C | - | High density lipoprotein cholesterol |
| HRP | - | Horse raddish peroxidases |
| IHD | - | Ischemic heart disease |
| kDa | - | kilo dalton |
| KO | - | Knockout mice |
| LDL-C | - | Low density lipoprotein cholesterol |
| Leu | - | Leucine |
| Lp (a) | - | Lipoprotein (a) |
| MI | - | Myocardial infarction |
| PAF | - | Platelet activate factor |
| PCR | - | polymerase chain reaction |
| PON | - | Paraoxonase |
| PTCA | - | Percutaneous trans coronary angioplasty |
| RCT | - | Reverse cholesterol transport |
| RFLP | - | Restriction fragment length polymorphism |
| ROS | - | Reactive oxygen species |
| SBP | - | Systolic blood pressure |
| Se | - | Selenium |

| | | |
|---|---|---|
| SNP | - | Small Nucleotide Polymorphism |
| SOD | - | Superoxide dismutase |
| WHO | - | World Health organization |

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**Novel cardiovascular risk markers Paraoxonase, Apolipoprotein A-1 (Apo A- 1) and Glutathione Peroxidase genotype -1 in Coronary Artery Disease.**

Wickramasinghege Dinushka Wickramasinghe

## ABSTRACT

Introduction: Coronary artery disease (CAD) is one of the major causes of mortality in both developed and developing countries. Oxidative stress has been demonstrated to have a role in pathogenesis of atherosclerosis. Reactive oxygen species (ROS) formed during oxidative stress result in oxidation of proteins and lipids of the cell membrane, leading to endothelial injury and microvascular dysfunction. Thus, the present study was designed to assess the relationship between severity of CAD as assessed by coronary angiography and Glutathione Peroxidase-1 (GPX-1), Paraoxonase-1 (PON-1), Apolipoprotein A-1 (Apo A-1) and GPX-1 genetic variants.

Objectives: This study was designed to investigate the relationship between GPX-1 variant, PON-1 and apoA-1 activity in healthy individuals and patients with CAD based on coronary angiographic severity scoring systems.

Methods: A case-control study of 75 patients (58 males, 17 females) with CAD (patients were selected from those awaiting coronary angiography) and age and sex matched 75 healthy volunteers as control subjects. Fasting venous blood samples were collected from all subjects for laboratory analysis of erythrocyte total GPX, erythrocyte GPX-1, serum PON-1 activity, Apo A-1 level and GPX-1 Pro198Leu polymorphisms.

Results: Data revealed that the serum PON-1 concentration, total erythrocyte GPX and erythrocyte GPX -1 activity were significantly ($p \leq 0.05$) low in patients when compared to controls. Paraoxonase-1 activity and Apolipoprotein A-1 levels did not show significant correlations with vessel, stenosis, and extent scores. Total erythrocyte GPX

and erythrocyte GPX-1 activities showed significantly strong inverse relationship with vessel, stenosis, and extent scores. Frequency distribution of GPX-1 Pro198leu (CT) genotype was significantly higher in patients (25.3%) when compared to controls (10.7%) ($\chi^2$ test =1.019). Results of genotype polymorphism in GPX-1 showed that the Leu198Leu (TT) genotype was not present in our study population. Interestingly, Pro198leu (CT) genotype showed a 2.84 fold risk for CAD [odds ratio 2.84 (95% CI 1.15 – 6.98), p = 0.019] in our study population. The Pro198Leu (CT) genotype carriers in subjects with age ≤ 50 years showed significantly higher (6.19 fold) risk for CAD compared to Pro198Pro (CC) genotype carriers in the same age group [odds ratio 6.19 (95% CI 1.1 – 34.3), p = 0.037)

Conclusion: Low serum PON-1 concentration, total erythrocyte GPX, and erythrocyte GPX -1 activity are independent risk factors for CAD. Decreased total GPX and GPX-1 activities are associated with increased severity of CAD. The Pro198Pro (CC) genotype is the most prevalent genetic variant of GPX-1 Pro198Leu polymorphism in the study population. However, Leu198Leu (TT) genotype not detected in our study population. The Pro198Leu (CT) genetic variant appeared to be the most significant predictor of CAD. Thus, this may have a future potential in early identification and management subjects with CAD.