Association of Glutathione Peroxidase 1 (GPX-1) activity with severity of coronary artery diseases

WD Wickramasinghe[1], H Peiris[2], LG Chandrasena[3], V Senaratne[4], and PPR Perera[5]

[1,2,5] *Department of Biochemistry, University of Sri Jayewardenepura, Sri Lanka*
[3] *Nawaloka Metropolis clinical laboratories, Nawaloka Hospitals PLC, Colombo, Sri Lanka*
[4] *Cardiology Unit, National Hospital, Colombo, Sri Lanka*

[1]dinushka15@gmail.com, [2]hempeiris@yahoo.com,[3]dgmnh@slt.net, [4]vajirasenaratne@gmail.com, [5]radeep_2005@yahoo.com>,([1]correspondence author)

***Abstract*— *Glutathione Peroxidase 1 (GPX-1) is a selenium-dependent enzyme with antioxidant properties. Studies have shown that the GPX-1 isoenzyme of Glutathione Peroxidase (GPX) appear to be a more sensitive indicator of oxidative stress in the pathogenesis of atherosclerosis and reperfusion injury. Limited evidence is available regarding the relevance of GPX-1 activity on human coronary artery disease and its severity. A case control study was carried out with age and sex matched patients with CAD (cases) and healthy subjects (controls). Patients awaiting coronary angiography were selected as cases. Blood samples were collected from the patients (n=60, mean age 54 ± 9 years, 45 Male, 15 Female) before coronary angiography. Sixty clinically healthy age and sex matched volunteers attending health screening programme were selected as controls. Angiogram findings were used to calculate vessel score which indicates the number of coronary arteries with ≥ 70% stenosis based on a method described by Sullivan et al in 1990. Angiogram positive cases had significantly low ($p < 0.05$) GPX-1 activity (mean 103.2 ± 33.0 U/L) than controls (mean 165.2 ± 43.0 U/L). The mean GPX-1 activity of cases were low in single vessel disease (101.2 ± 32.7 U/L) compared to those with double vessel disease (102.1 ± 33.3 U/L) and triple vessel disease (111.2 ± 37.1 U/L) respectively. There was a significant correlation between GPX-1 activity and vessel score groups ($r = 0.536$, $p = 0.000$). This study reveals that erythrocyte GPX-1 activity would be helpful in assessing the severity of CAD.*

***Keywords*— Coronary artery disease, Glutathione Peroxidase 1, Vessel score**

I. INTRODUCTION

Coronary artery disease (CAD) is the commonest heart disease among adults in the age group 45 and above. It is well established that several cardiovascular risk factors, including hypertension, hypercholesterolemia, and diabetes are associated with oxidative stress (Wassmann et al, 2004). Oxidative stress has been demonstrated to have a role in pathogenesis of atherosclerosis in past studies (Forgione et al, 2002). 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. Over production of ROS is neutralized by various antioxidants. Antioxidants inhibit atherogenesis and improve vascular functions by various mechanisms. The vascular endothelium is protected from oxidant stress by expressing enzymes with antioxidant properties such as the selenocysteine-containing protein Glutathione Peroxidase 1 (GPX-1). GPX-1 is the first selenoprotein identified in mammals, is present in both cytosol and mitochondria as a key antioxidant enzyme in many cells, including the vascular endothelium. The activity of GPX-1 is widespread, with highest levels in liver, kidney, heart and it has been identified as an important antiatherogenic enzyme (Blankenberg et al, 2003). As GPX-1 plays a central role in protecting cells from ROS, its deficiency may lead to an increase of oxidant stress in the cell (Forgione et al, 2002). GPX-1 uses reduced glutathione to convert hydrogen peroxide (H_2O_2) to water and lipid peroxides to their respective alcohols. Thus, GPx-1 deficiency may lead to an increase of oxidant stress in the cell.

Several studies have shown that a variety of oxidant and antioxidant enzymes play an important role in the pathogenesis of vascular disease, and low levels of GPX 1 activity have been found to be an independent risk factor for cardiovascular events in patients with coronary artery disease (Blankenberg et al, 2003). In vitro data and studies in animal models suggest that GPX1 may protect arteries from atherosclerosis. However, information available on the association of severity of CAD and GPX-1 in human is limited.

II. MATERIALS AND METHODS

A case-control study was carried out with 60 CAD patients (45 male, 15 female) as cases and age and sex matched 60 healthy volunteers as controls. Angiographically proven CAD Patients aged 45 to 75 years were selected from those awaiting coronary angiography at National Hospital, Sri Lanka and Nawaloka Hospital PLC, Colombo. Controls were selected from persons attending the Family Practice Centre, University of Sri Jayewardenepura for their health screening programme. Inclusion criteria for normal controls were absence of any clinical or diagnostic

evidence of heart disease, kidney disease, liver disease, diabetes mellitus or cancer.

Blood samples of the patients and control individuals were taken after a 10 hour fast. Five milliliters blood was collected to a vaccutainer containing lithium heparinized anticoagulant. 0.5 milliliters of blood was washed 3 times with 4 volumes of isotonic saline and packed red cells were used with 1ml of deionized water to prepare hemolysate for GPX1 activity.

A. Laboratory analysis of GPX-1 activity

Plasma GPX 1 was measured indirectly by a coupled reaction with Glutathione Reductase (GR) as described by Paglia and Valentine (1967). Glutathione Peroxidase catalyzes the reduction of hydrogen peroxide (H2O2), oxidizing reduced glutathione (GSH) to form oxidized glutathione (GSSG). GSSG is then reduced by glutathione reductase (GR) and β-nicotinamide adenine dinucleotide phosphate (NADPH) forming NADP+ (resulting in decreased absorbance at 340 nm) and recycling the GSH. Because GPX is limiting, the decrease in absorbance at 340 nm is directly proportional to the GPX-1 concentration. GPX-1 assay was carried out as an ELISA method by using Bio-Rad 680 microplate reader.

B. Calculation of vessel score

Angiogram findings were interpreted by a consultant cardiologist. *Severity of CAD was calculated* based on the scoring system described by Sullivan et al 1990. The scoring system is based on the extent of stenosis in identified four coronary arteries. Left main coronary artery, left anterior descending artery, left circumflex artery and right coronary artery were considered in scoring the vessels score and if ≥ 70% stenosis in lumen given a score 1 for each while left main coronary artery with ≥ 70% stenosis was considered as single vessel disease (score 1). Therefore, vessel score on a scale ranged from 0 to 3.

C. Statistical analysis

Statistical analysis was performed using "SPSS 16.0 for Windows". Data was expressed as "mean ± standard deviation (SD)" and/or percent (%). Descriptive statistics were given for numerical variables and frequency tables for categorical variables. Pearson Correlation test was used to determine the relationship between variables. Student t-test was used for the numerical data. A $P \leq 0.05$ was considered statistically significant.

Written informed consent was obtained from each subject following a detailed explanation of the objective and protocol of the study. Ethical approval for the study was obtained from the Ethics Review Committee (ERC) of Faculty of Medical Sciences, University of Sri Jayewardenepura.

III. RESULT

The total study population of 120 volunteers had a mean age of 54 ± 10 years and each group consisted of 45 (75%) males and 15 (25%) females. Results revealed that angiogram positive cases had significantly low ($p < 0.05$) GPX-1 activity (mean 103.2 ± 33.0 U/L) than controls (mean 165.2 ± 43.0 U/L). The mean GPX-1 activity of cases were low in single vessel disease (101.2 ± 32.7 U/L) when compared to those with double vessel disease (102.1 ± 33.3 U/L) and triple vessel disease (111.2 ± 37.1 U/L) respectively. There was a significant correlation between GPX-1 activity and vessel score groups ($r = 0.536$, $p = 0.000$).

IV. DISCUSSION

GPX-1 is the most abundant selenium containing peroxidase and a key antioxidant enzyme in many cell types including vascular endothelial cells. Previous studies have shown that GPx1- over expressing mice were more resistant to developing atherosclerosis compared to GPX-1 knockout mice (Cheng et al, 1998). Suggesting the GPX-1 is likely to be an important antiatherogenic enzyme (Cheng et al, 1998). In patients with CAD, the low activity of erythrocyte GPX-1 is associated with an increased risk of cardiovascular events independent of traditional risk factors, and an increase in GPX-1 activity reduces cardiovascular risk (Blankenberg et al, 2003). Previous Studies indicate that a decrease in erythrocyte GPX levels are related to increased cardiovascular risk depending on the degree of atherosclerosis. In our study, erythrocyte GPX 1 levels were significantly lower in the CAD patients when compared with a group of healthy individuals (Figure 1 shows GPX-1 levels between patients with CAD and controls). This study which examined the relationship between GPX-1 activity and severity of coronary artery disease shows that GPX-1 activity is low in patients with single vessels disease compared to patients with double and triple vessel disease (Figure 2 shows association of GPX-1 levels in the three vessel score groups). Therefore, this study shows that GPX-1 activity and the Sullivan's vessel scoring system gives a better indication of the severity of CAD and has the potential to predict the severity of CAD.

V. CONCLUSION

Erythrocyte GPX-1 may be a helpful indicator for assessing the severity of CAD based on vessel scoring system.

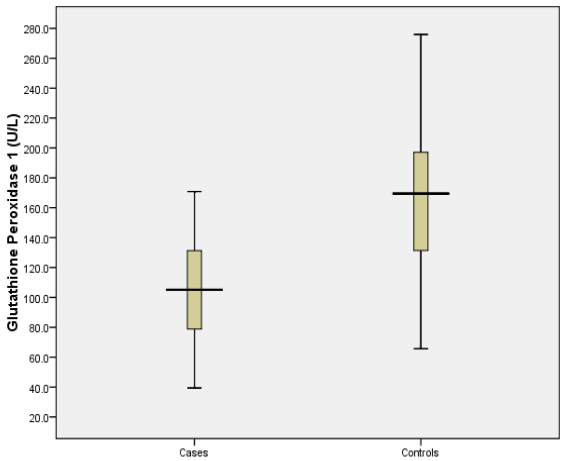

Fig 1. Glutathione Peroxidase 1 levels between cases and controls

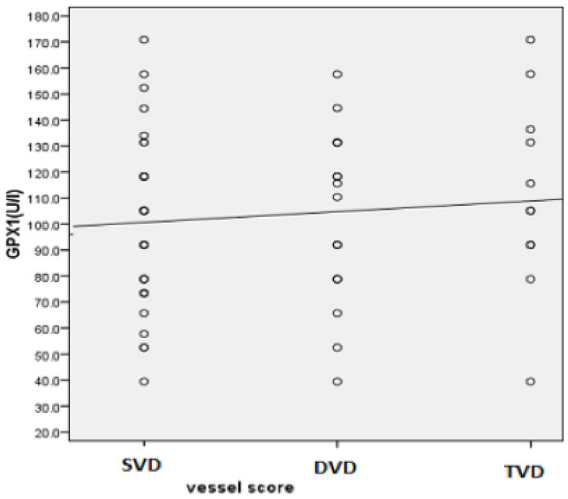

Fig 2. Glutathione Peroxidase 1 levels in vessel score groups. (SVD- Single Vessel disease, DVD- Double Vessel Disease, TVD- Triple Vessel disease)

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