Reduced Glutathione Peroxidase Activity Predicts Increased Cardiovascular Risk Following an Acute Coronary Syndrome

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Abstract

Background
Glutathione peroxidase (GPx) is an antioxidant enzyme that comprises part of the primary defence against oxidative stress. Deficiencies in GPx have been associated with progression of atherosclerosis and arterial thrombosis. However, the relationship between GPx activity and clinical outcomes in acute coronary syndromes (ACS) remains controversial. This study examined the relationship between plasma GPx activity and subsequent major adverse cardiovascular events (MACE) in ACS patients.

Methods
In this prospective cohort study, 262 ACS patients had their plasma GPx activity measured using a colourimetric enzyme assay. Patient demographics, clinical variables and MACE, a composite of death, non-fatal myocardial infarction, ischaemic stroke and acute heart failure, were collected.

Results:
At 1 year follow-up, 34 (13%) patients experienced MACE. When the MACE rate was examined by quartiles of GPx activity, a significant decrease in MACE was demonstrated with increasing quartiles of GPx activity (P = 0.04). The event rate for patients in the lowest quartile of GPx activity was 19.6% compared to 7.9% for the upper quartile. Levels of GPx activity were significantly lower in diabetic patients compared to non-diabetic patients (P = 0.02), and lower in males compared to females (P = 0.03). GPx activity was a moderate predictor of MACE by receiver operator characteristic analysis with an area under the curve of 0.62 (P = 0.02).

Conclusions
We have demonstrated that lower levels of GPx activity are associated with an increased risk of MACE at 1 year following an ACS. Low levels of GPx activity may represent a decreased defence against oxidant-mediated cardiovascular damage.

Keywords: Glutathione peroxidase; acute coronary syndromes; cardiac event; oxidative stress

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Introduction

Glutathione peroxidase (GPx) is a family of antioxidant enzymes that reduce reactive oxygen species (ROS), thereby constituting a primary defence system against oxidative stress. Of the five characterised isoforms of GPx found in mammals, GPx1 is the predominant intracellular form and GPx3 is the predominant form found in the extracellular space[1]. Experimental studies have demonstrated that deficiencies in GPx1 activity result in abnormal vascular and cardiac structure and function[2]. Deficiencies in the GPx3 enzyme lead to a pro-thrombotic state in mouse models[3]. Clinical case studies have demonstrated an association between decreased levels of plasma GPx activity and familial arterial thrombosis[4]. In stable coronary artery disease, patients with lower levels of GPx1 activity have a higher incidence of major adverse cardiovascular events (MACE)[5].

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On the basis of these observations, we hypothesised that in the context of acute coronary disease, patients with lower levels of GPx activity would have a reduced antioxidant defence capability and in turn, would have worse clinical outcomes. This would be consistent with the majority of the literature examining the relationship between GPx activity and the progression of coronary artery disease that indicates an inverse relationship between the two parameters[6]. The largest study conducted in acute coronary syndrome (ACS) patients to date, however, indicates that patients with higher levels of plasma GPx activity have worse clinical outcomes[7], suggesting that rather than representing a protective capability, GPx activity may increase in response to the production of ROS and therefore be indicative of higher levels of oxidative stress.

The aim of the present study was to address the apparent discrepancy in the literature surrounding GPx activity and clinical outcome following an ACS. We prospectively examined the relationship between plasma GPx activity and MACE at 1 year in an ACS patient population.

Methods

Study population

We prospectively studied a group of 262 patients presenting to Wellington Regional Hospital with an ACS between January 2012 and October 2012 in whom an early invasive approach of coronary angiography ± percutaneous coronary intervention (PCI) was planned. An ACS was defined as symptoms suggestive of myocardial ischaemia lasting > 10 minutes, and either troponin elevation or ≥1 mm of new ST segment deviation or ≥1 mm T wave inversion on an electrocardiogram in at least two contiguous leads. Patients were adequately pre-treated with dual anti-platelet therapy. Exclusion criteria included cardiogenic shock, a platelet count of less than 100 x 109/L, a known platelet function disorder, administration of a thrombolytic agent within 24 hr of enrolment or administration of a glycoprotein IIb/IIIa receptor antagonist within the week prior to enrolment. The study was reviewed and approved by the Central Regional Ethics Committee, and each patient gave informed written consent.

Data collection

Patient demographics, clinical characteristics, medications, procedural variables and clinical management were collected prospectively from review of the medical records and cardiac catheterisation database.

Follow-up and MACE

Clinical follow-up was collected by telephone at 30 days and 1 year. Where necessary, a review of case notes was performed and the appropriate general practitioner contacted to further classify clinical outcomes. The primary endpoint was a composite of MACE that included death, nonfatal myocardial infarction (MI), nonfatal ischaemic stroke, stent thrombosis and new heart failure presentation.

Blood collection

Whole blood samples were collected in tubes anti-coagulated with sodium citrate (0.109M, BD Vacutainer; New Jersey, USA) from a peripheral vein using a 21-gauge needle before angiography or in the cardiac catheterisation laboratory from the arterial sheath immediately after insertion and prior to heparin administration. Plasma was separated from the cellular components by centrifugation at 1500 g for 12 min at 4°C. Aliquots of plasma were stored at -80°C for subsequent analysis of antioxidant activity.

Glutathione peroxidase activity assay

GPx activity kits (Enzo Lifesciences; New York, USA) were used as per manufacturer's instructions using a colourimetric assay. The experimental protocol was based on a coupled reaction of GPx with the reduction of oxidised glutathione by glutathione reductase using NADPH. The oxidation of NADPH to NADP+ accompanies a decrease in absorbance at 340 nm that is proportional to total GPx activity found in the plasma sample. GPx activity was defined as nanomoles of NADPH consumed per minute and expressed as units per mL of plasma. The intra-assay coefficient of variance was 7.3%, and the inter-assay coefficient of variance was 9.9%.

Statistical Analysis

Continuous variables were reported as the mean ± standard deviation (SD), and categorical variables were reported as frequencies and percentages. Statistical tests to compare continuous variables with the rate of MACE were carried out using a Student's unpaired t-test and categorical variables were analysed using chi-square tests. Relationships between continuous parameters were determined by the Pearson's correlation coefficient. Where univariate factors were associated significantly with MACE, multinominal logistic regression was performed to determine independent associations with MACE. A receiver operator curve was used to examine the relationship between GPx activity levels and MACE. GPx activity was divided into quartiles in order to examine the rate of MACE using a linear-by-linear association test. Differences in values corresponding to $P < 0.05$ were taken as statistically significant. All statistical analyses were carried out in either GraphPad Prism Software v.6 (GraphPad Software Inc; California, USA) or SPSS v.22 (IBM; New York, USA).

Results

Baseline characteristics

The demographic data and clinical characteristics of the 262 ACS patients are summarised in Table i. The study population had a mean age of 63 ± 10 years with 69% being male. The clinical presentation was ST-segment elevation MI (STEMI) in 23%, non-STEMI (NSTEMI) in 71% and unstable angina (UA) in 7%. The mean Grace Score on admission was 100 ± 24. The clinical management of the study group was as follows: PCI in 50%, coronary artery bypass grafting (CABG) in 14% and medical management in the remaining 34%.

Patient outcome

At 1 year follow up, 34 (13%) patients experienced MACE. This included 10 deaths (3.8%) all from cardiovascular causes. Nonfatal MI occurred in 11 patients (4.2%), and ischaemic stroke in 4 patients (1.5%). The rate of stent thrombosis was relatively low, occurring in 2 patients (0.8%) during the follow-up period. A further 7 patients (2.7%) were admitted with acute heart failure presentations.
When comparing the patients with MACE to the patients without MACE (Table i), those with MACE were older, more likely to have a history of hypertension, dyslipidaemia, diabetes and renal dysfunction, and have higher Grace Scores.

Antioxidant activity

The mean plasma level of GPx activity in the ACS population was 123 ± 32 U/mL. Patients who experienced MACE were found to have significantly lower levels of plasma GPx activity compared to patients who did not experience MACE ($P = 0.03$)

Table 1. Baseline characteristics of the ACS study population

Characteristic	ACS patients (n=262)	MACE Group (n=34)	No MACE Group (n=228)	P value
Male	180 (69)	26 (76)	154 (68)	0.29
Age (years)	63 ± 10	66 ± 11	62 ± 10	0.02
BMI (kg/m^2)	29.5 ± 5.8	29.9 ± 7.2	29.5 ± 5.5	0.64
Risk Factors				
Hypertension	175 (67)	31 (91)	144 (63)	0.001
Dyslipidaemia	182 (70)	31 (94)	151 (66)	0.003
Diabetes	66 (25)	18 (52)	48 (21)	0.001
Current Smoker	54 (21)	8 (25)	46 (20)	0.65
Renal Dysfunction	17 (7)	6 (17)	11 (5)	0.005
Clinical Presentation				
STEMI	59 (23)	4 (12)	55 (24)	
NSTEMI	185 (71)	28 (82)	157 (69)	0.24
Unstable Angina	18 (7)	2 (6)	16 (7)	
Grace Score	100 ± 24	111 ± 27	99 ± 24	0.006
Clinical Management				
Medical Management	95 (36)	15 (44)	83 (36)	
PCI	131 (50)	12 (35)	119 (52)	0.12
CABG	35 (14)	7 (21)	26 (11)	
Antioxidant Enzyme Activity				
GPx activity	123 ± 32.4	112 ± 33.5	125 ± 32.0	0.03

Legend to table 1: Continuous variables are expressed as mean ± SD, categorical variables are expressed as frequencies and (percentages). Abbreviations: ACS – acute coronary syndrome; BMI – body mass index; CABG – coronary artery bypass grafting; GPx – glutathione peroxidase; MACE- major adverse cardiovascular events; NSTEMI – non-ST elevation myocardial infarction; PCI – percutaneous coronary intervention; STEMI – ST-elevation myocardial infarction.

GPx activity was found to be significantly lower in diabetic patients (115 ± 32 U/mL) compared to non-diabetic patients (126 ± 32 U/mL) (P = 0.02), and significantly lower in males (120 ± 33.5 U/mL) compared to females (129 ± 29 U/mL) (P = 0.03). The remaining cardiovascular risk factors did not appear to correlate to levels of GPx activity.

Predictive value of GPx activity for MACE

To assess the predictive value of plasma GPx activity at identifying patients at increased risk of MACE, a receiver operator characteristic (ROC) curve analysis was conducted. GPx activity was found to be a moderate predictor of MACE with an area under the curve of 0.62 that was significantly different from 0.5 (P = 0.02) (Figure 1). The curve however lacked a single cut-point that would indicate an optimal level of GPx activity that was predictive of MACE risk.

When the MACE rate was examined by quartiles of GPx activity, a significant decrease of MACE was demonstrated across the four quartiles (P = 0.04). The event rate for patients in the lowest quartile of GPx activity (19.6%) was approximately 2.5 times higher than that for patients in the upper quartile of GPx activity (7.9%) (Figure 2).

In a multivariate model (multinominal logistic regression) where all univariate predictors of MACE were incorporated, including GPx activity, age, hypertension, dyslipidaemia, diabetes and renal dysfunction, only diabetes was significantly associated with MACE (odds ratio 2.5, 1.1-5.8, P = 0.02).

Discussion

To the best of our knowledge, this is the largest prospective cohort study to examine the relationship between plasma GPx activity levels and the occurrence of MACE in patients with ACS. We have demonstrated significantly lower plasma levels of GPx activity in patients who experienced MACE within 1 year compared to those who did not. Our ROC curve analysis demonstrated a modest, but significant relationship between plasma GPx activity and MACE, although a clear cut-off point at which GPx activity optimally predicts risk could not be defined. The MACE rate in patients with GPx activity in the lowest quartile was 2.5 times higher compared to the MACE rate found in patients in the highest quartile of GPx activity.

We observed a modest relationship between plasma GPx activity and MACE, with patients in the lowest quartile of GPx activity experiencing highest MACE rates. This observation is consistent with the results reported by Blankenberg et al in which an increased rate of MACE in stable coronary artery disease was observed in those patients in the lowest quartile of GPx activity[5]. A number of other clinical studies have suggested a relationship between low levels of GPx activity

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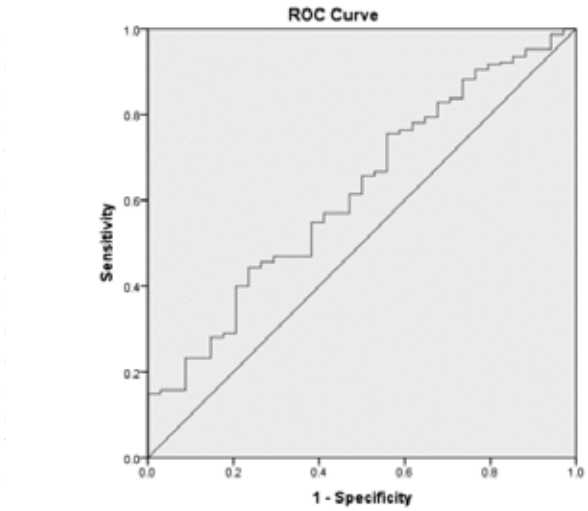

Figure 1. ROC curve analysis of GPx activity and MACE. The ROC curve is a graph of sensitivity (y-axis) vs. 1-specificity (x-axis). Although the area under the curve was significantly different from 0.5 (0.62, P <0.05), a single cut-off value corresponding to maximum sensitivity with a high specificity could not be determined for plasma GPx activity and prediction of MACE.

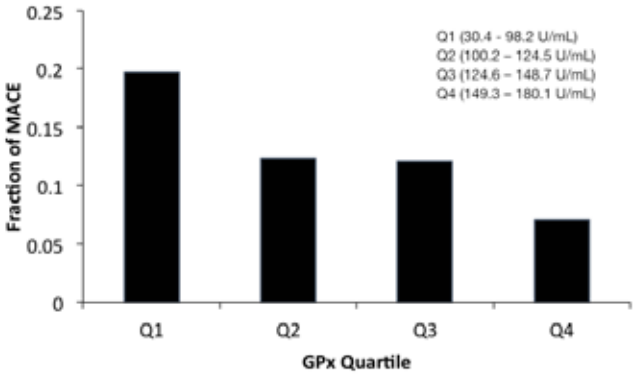

Figure 2. MACE rate by GPx quartile. The fraction of MACE rate as observed by plasma GPx activity quartile. MACE rate significantly decreased across the GPx quartiles, with the highest fraction of MACE occurring in the lowest quartile of GPx activity. The lowest MACE fraction occurred in the upper quartile of GPx activity. Data plotted from all 262 ACS patients, P <0.05 Linear-by-Linear association.

A recent meta-analysis by Flores-Mateo et al[6] examined 32 case-controlled studies and 2 prospective cohort studies that investigated the relationship between GPx activity and the development of coronary artery disease, and reported that for every 1 standard deviation increase in the level of GPx activity, the pooled odds ratio for progression of disease was 0.51 (95% confidence intervals 0.35 - 0.75). While this would again be consistent with our findings that lower levels of GPx activity were associated with worse outcomes, the authors noted that there was "substantial heterogeneity in the direction and magnitude of the association" between GPx activity and clinical outcomes[6].

Our results are in direct contrast to a study by Garcia–Pinilla et al[7]. This study examined 137 ACS patients and reported that the 2-year MACE rate was significantly higher in patients whose GPx activity was above the 50th percentile. It is not clear how to explain this finding in light of our reported results; however, the two studies differed in methodology. Our study collected blood samples prior to angiography, whereas Garcia-Pinilla et al collected blood following angiography and revascularisation. It is likely that GPx activity levels are highly dynamic during an ACS presentation, but how variable GPx activity levels are over time, and what influence revascularisation has on this has not been examined.

A possible explanation for the findings in our study is that high levels of GPx activity may provide a greater protection against oxidative stress, and therefore able to protect against adverse cardiovascular outcomes. The alternative, as suggested by Garcia-Pinilla et al, is that high levels of GPx activity may occur in response to a greater oxidative stress load experienced by patients, and therefore an indirect marker of an adverse risk profile. Due to the volatile nature of ROS, and their relatively short half-lives, measurement of these molecules in a clinical setting can be challenging to researchers. Currently there is not a widely accepted gold-standard marker for ROS in the circulation; therefore, selecting an appropriate marker of oxidative stress to relate to GPx activity remains challenging to researchers. This makes it difficult to examine the dynamic relationship between ROS and GPx activity during an ACS. The ability to quantify the level of oxidative stress occurring due to a shift in the net redox balance may shed light on the apparent discrepancy between the study by Garcia-Pinilla et al and the current study.

Diabetic patients in our study had significantly lower levels of GPx activity compared to non-diabetic patients. Diabetic patients have been previously described to have a higher oxidative stress load[11,12], along with lower levels of GPx activity[13,14]. In addition, males were found to have significantly lower levels of GPx activity when compared to females. It is possible that these factors may contribute to the inverse relationship observed between GPx activity and MACE; however, the present study was not powered to examine these factors in a multivariate model. No other relationships were observed between GPx activity and other clinical variables.

Limitations

While GPx activity was a univariate predictor of MACE, it was not significantly related to MACE in a multivariate model of analysis. Although the size of our study population of 262 ACS patients was not powered to examine some of the observed relationships in a multivariate model, it was designed to give sufficient power to examine univariate relationships. To the best of our knowledge this study is still the largest ACS cohort prospectively examining the relationship between GPx activity and clinical outcomes.

Our blood samples were taken at a non-standardised time point after patients were admitted to hospital following an ACS presentation. It is thought that the magnitude and time course of antioxidant enzyme changes may be affected by the severity of the acute event and subsequent therapeutic intervention such as reperfusion[15,16]. Closer examination of the dynamic change in GPx activity during an ACS event will be crucial to understand how it is affected by sampling time and interventional strategies such as revascularisation. If GPx activity is to be used as a risk marker it is essential to understand how GPx activity changes over the course of an ACS event.

A further limitation of the current study is that the GPx activity assays used cannot differentiate between the various isoforms of GPx that may be present in the plasma. Although GPx3 is reported to be the predominant isoform in the plasma, it is possible that other GPx isoforms secreted from cellular locations are also contributing to total activity[17]. However, the purpose of this study was to measure global GPx activity present in the circulating plasma and relate it to the risk of MACE.

Conclusion

This study demonstrates a significant inverse relationship between plasma GPx activity and the rate of MACE in ACS patients. These findings suggest that patients with lower levels of plasma GPx activity are at an increased risk of developing adverse clinical events within 1 year following an ACS event, possibly due to a decreased defence against oxidant-mediated damage to the cardiac system. Future studies focusing on even larger cohorts of acute coronary patients and determining what factors influence the variable levels of GPx activity are warranted.

Declarations of Interest

The authors declare that there is no conflict of interests

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