Clinica Chimica Acta 489 (2019) 177–182

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Comparison of glutathione peroxidase-3 protein expression and enzyme bioactivity in normal subjects and patients with sepsis

Wei-Ju Lee, Yen-Ling Chen, Yi-Wen Chu, Du-Shieng Chien*

TaiRx, Inc., Taipei 11502, Taiwan

ARTICLE INFO

Keywords:
GPx-3 bioactivity
GPx-3 protein concentration
Sepsis
Biomarker
Selenium

ABSTRACT

Background: Serum glutathione peroxidase-3 (GPx-3) is known as a key selenoprotein with antioxidant properties. GPx-3 deficiency has been associated with sepsis. The objectives of this study are (1) to compare the GPx-3 protein concentrations and GPx-3 bioactivity in normal healthy subjects and septic patients, and (2) to evaluate the relationship between GPx-3 bioactivity and its protein concentration.

Methods: Serum samples were collected from 50 normal healthy subjects and 70 septic patients. The reliable bioanalytical methods for GPx-3 protein concentration and bioactivity in human serum were developed and validated. Analyses of GPx-3 bioactivity and GPx-3 protein concentration were then performed.

Results: Geometric mean GPx-3 bioactivity was 78.13 U/l for patients with sepsis, significantly lower than normal subjects with 108.21 U/l ($p < 0.0001$). Similarly, the GPx-3 protein concentration was significantly lower in patients with sepsis than in normal subjects, with the mean GPx-3 value of 0.78 vs 3.10 μg/ml, respectively ($p < 0.0001$). A positive correlation was observed between the GPx-3 bioactivity and its corresponding protein concentration in septic serum samples ($R = 0.74$, $p < 0.0001$), regardless of gender or age difference.

Conclusion: These findings suggest that the decrease in GPx-3 bioactivity observed in the septic patients was resulted from the significant sepsis-related decline of GPx-3 protein concentrations.

1. Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. Sepsis-induced organ dysfunction may be occult. Oxidative stress in septic patient caused by inherent inflammatory response to pathogens could lead to initiate lipid peroxidation, DNA damage, deterioration of mitochondrial function, and further develop into organ dysfunction and failure [2].

The identification of altered components as direct and indirect biomarkers in these complex reactions has gained much attention in development of medical means [3,4]. Among these researches, circulated glutathione peroxidase (GPx) appears to be the most promising biomarker for evaluating the oxidative stress in septic patients. As an antioxidant enzyme, GPx plays a critical role in protecting the human major vital tissues and organs from oxidative damage. The main biochemical function of GPx is to catalyze the reduction of hydrogen peroxide, organic hydroperoxides, and lipid peroxides by converting reduced glutathione (GSH) to oxidized glutathione (GSSG). The bioactivity of GPx-3 has been reported to be correlated inversely with the mortality and disease severity of patients with sepsis [4].

Selenium (Se) is essential for the unique activity of GPx and presents in the form of selenocysteine in the catalytic site, which is therefore known as selenoprotein [4]. The selenoprotein GPx family has several subspecies differed by their tissue location and substrate specificity. For example, GPx-1 is found in the cytoplasm of nearly all mammalian tissues, and GPx-3 herein is an extracellular glycoprotein, abundantly found in serum or plasma [5].

Sepsis has been found to be accompanied with low GPx-3 bioactivity in several early clinical studies by comparing the baseline GPx-3 activity in septic patients against healthy volunteers [6–9]. However, the mechanism of this phenomenon remains unclear. When sepsis occurs, the accumulation of increased concentrations of peroxides resulting from GPx-3 inactivation under oxidative stress may induce GPx expression and restore the GPx protein concentration to protect against cell damage [10]. On the other side, low systemic Se concentrations were found in many inflammation-related diseases such as sepsis, and the reduced GPx-3 bioactivity was strongly associated with the low availability of Se [6,7,11–15]. Until now, very few studies have been

Abbreviations: GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; Se, selenium; GR, glutathione reductase; STREP-HRP, horseradish peroxidase labeled streptavidin; ROS, reactive oxygen species

* Corresponding author at: 7F-5, No. 66, San-Chung Road, Nankang Business Park, Taipei 11502, Taiwan.
E-mail address: dschien@trx.com.tw (D.-S. Chien).

http://dx.doi.org/10.1016/j.cca.2017.10.031
Received 25 July 2017; Received in revised form 25 September 2017; Accepted 30 October 2017
Available online 31 October 2017

conducted to evaluate the change of GPx-3 protein concentration along with reduced GPx-3 bioactivity in septic patients. Also, the effects of GPx-3 protein concentration, gender, and age of septic patients on GPx-3 bioactivity have not yet been well evaluated.

2. Materials and methods

2.1. Sources of serum samples

The human blood samples were collected from 50 normal healthy subjects and 70 patients with sepsis in the United States by Bioreclamation IVT during the period of November 2015–August 2016. The inclusion criteria of septic patients were based on the then international sepsis definitions, sepsis-2 or sepsis-3 [1,16]. For each subject, the serum was separated from the blood sample using standard centrifugation technique and kept frozen at ≤ − 70 °C until analysis.

2.2. Measurement of GPx-3 bioactivity in human serum

GPx-3 enzymatic activity in serum was measured using coupled enzyme system by a GPx activity colorimetric assay kit (BioVision). Briefly, the cumene hydroperoxide reduction and oxidation of GSH catalyzed by serum GPx-3 was coupled to the reduction of GSSG and oxidation of NADPH by glutathione reductase (GR). The decrease of NADPH measured by spectrometry is proportional to the GPx-3 bioactivity.

Approximately 50 µl of undiluted serum sample was added to 40 µl of the reaction mix solution, containing NADPH, GSH and GR solutions and assay buffer, mixed well, and incubated for 15 min to deplete all GSSG in the sample. All test samples were centrifuged at 4000 × *g* for 10 min at 4 °C to remove insoluble particles. The reaction was initiated by adding 10 µl of cumene hydroperoxide solution, then measured the absorbance at 340 nm and 25 °C on the SpectraMax® Plate Reader every minute for 20 min using kinetic reading. Data collected from a 5-min interval between 1 and 6 min within a linear reaction curve was used for the calculation of GPx-3 bioactivity. The NADPH standard curve with the coefficient of determination (R^2) > 0.999 was applied to calculate the value of GPx-3 bioactivity from OD450. One unit of GPx-3 bioactivity is defined as the amount of enzyme that causes the oxidation of 1.0 µmol of NADPH to $NADP^+$ under the assay kit condition per minute at 25 °C. All samples were run in duplicate.

The accuracy (%RE) of the assay for normal serum and septic serum were − 6.71% and − 11.90%, respectively. The intra-run CVs for both normal serum and septic serum was ~0.6–1.1%. The inter-run CV% for normal serum and septic serum were 11.2% and 5.3%, respectively.

2.3. Measurement of GPx-3 protein concentration in human serum

A commercial AdipoGen® human GPx-3 ELISA was used to determine the GPx-3 protein concentration in human serum samples. Serum samples were incubated in the wells of a microplate precoated with polyclonal antibody specific for GPx-3 protein. GPx-3 protein was then detected with biotinylated polyclonal antibody and horseradish peroxidase labeled streptavidin (STREP-HRP) using 3,3′,5,5′-tetramethylbenzidine as HRP substrate. The intensity of the color reaction measured using the SpectraMax® at 450 nm is directly proportional to the protein concentration of GPx-3 in the serum sample.

Procedures of GPx-3 protein concentration measurement in serum were conducted based on the instructions from the manufacturer with modifications. Briefly, the calibration curve was generated in the range of concentrations from 1 to 32 ng/ml. The dilution linearity across the dilution factors from 1:10 to 1:320 was confirmed, with the accuracy (%RE) within ± 25% of the nominal value. The serum samples were first diluted 80 times to measure their GPx-3 protein concentration. For those serum samples not falling into the calibration curve range, re-dilutions will be needed. All samples were run in duplicate.

The coefficient of determination (R^2) of the quadratic calibration curve was high (> 0.999). The lower limit of quantitation (LLOQ) of serum GPx-3 protein concentration in this procedure was determined to be 0.01 µg/ml. The assay precision (%CV) and accuracy (%RE) were determined separately in serum samples collected from normal healthy subjects and septic patients. The assay validation results showed that the intra-run and inter-run precision were 3.3–15.7% and 10.1–23.4%, respectively, while the intra-run and inter-run accuracy were − 23.9–19.9% and − 14.0–11.0%, respectively. There were no differences in the assay accuracy and precision between the normal subjects and septic patients.

2.4. Statistics

The serum GPx-3 protein concentrations and bioactivity results were expressed as geometric mean with standard error of the mean (SEM). Descriptive statistics were used to summarize demographic variables including gender, age, and race. For comparison of GPx-3 bioactivity and GPx-3 protein concentration between the normal healthy subjects and patients with sepsis, significant differences ($p < 0.05$) were determined by two tailed, unpaired, Student's *t*-test. Pearson correlation coefficients were calculated to determine the correlation between the GPx-3 bioactivity and its corresponding protein concentration in human serum.

Simple linear regression analysis was used to determine the univariate association of GPx-3 bioactivity with GPx-3 protein concentrations, gender and age. Sequential multiple linear regression model building method was used to adjust for the gender and age differences in the examination of the association of GPx-3 bioactivity with its protein concentration. In this hierarchical regression analysis, these covariates were added one at a time to examine the effect of each additional covariate on the association between GPx-3 bioactivity and GPx-3 protein concentration.

3. Results

3.1. Demographic profile

Demographic information including age, gender, and race of the blood donors was obtained from vendor and summarized in Table 1. It should be noted that part of the age and race information was not available for some donors. Overall, 50% of normal subjects and 57% of septic patients were male. The mean ages of 30 normal subjects and 70 septic patients were 41.2 and 61.6, respectively. Of the 30 normal subjects, 20 subjects were African American, 6 subjects were Hispanic,

Table 1
Demographic summary for the serum samples from normal subjects and patients with sepsis.[a]

	Normal subjects		Patients with sepsis	
	Number of available observations	Ratio (%)	Number of available observations	Ratio (%)
Age (y)[b]	30	41.2 ± 10.0	70	61.6 ± 16.6
Gender (male/female)	50	50/50	70	57/43
Race	30		40	
Caucasian		10.0		85.0
African American		66.7		10.0
Hispanic		20.0		5.0
Asian		3.3		0.0

[a] Only portion of total serum samples were available for demographic analysis.
[b] Age was expressed as arithmetic mean ± SD.

Table 2
Comparison of GPx-3 bioactivity, GPx-3 protein concentration, and specific activity of GPx-3 in the serum samples from normal subjects and patients with sepsis.

	Normal subjects		Patients with sepsis	
	Geometric mean	SEM	Geometric mean	SEM
GPx-3 bioactivity (U/l)	108.21	1.04	78.13	1.05
GPx-3 protein concentration (μg/ml)	3.10	1.11	0.78	1.17
GPx-3 specific activity (mU/μg)	34.96	1.09	99.92	1.14
Number of samples	50		70	

SEM, standard error of the mean.

3 subjects were Caucasian, and 1 subject was Asian. The 40 septic patients with available information on race were mainly Caucasian (85%), with African American and Hispanic 10% and 5%, respectively.

3.2. Comparison of GPx-3 bioactivity and protein concentrations

The GPx-3 bioactivity and GPx-3 protein concentrations in human serum samples collected from normal subjects and patients with sepsis were determined with validated assays and are listed in Supplemental Tables 1 and 2, respectively. The serum GPx-3 bioactivity ranged from 47.92 to 164.68 U/l in normal subjects and from 29.04 to 152.21 U/l in septic patients. Geometric mean serum GPx-3 bioactivity in septic patients was 78.13 U/l, approximately 38% lower than that of normal subjects (108.21 U/l) (Table 2). The results showed that the serum GPx-3 in septic patients was significantly lower than that in normal healthy subjects ($p < 0.0001$) (Fig. 1A).

The serum GPx-3 protein concentrations ranged from 0.22 to 8.56 μg/ml in normal subjects (Supplemental Table 1) and from 0.01 to 6.56 μg/ml in patients with sepsis (Supplemental Table 2). Geometric mean serum GPx-3 protein concentration in patients with sepsis was 0.78 μg/ml, only one-fourth of the concentration in normal subjects, 3.10 μg/ml (Table 2). Significant difference of GPx-3 protein concentration ($p < 0.0001$) was shown between normal healthy subjects and septic patients (Fig. 1B).

Specific GPx activity values of serum GPx-3, normalized by its protein concentration, in normal subjects and septic patients were 34.96 and 99.92 mU/μg, respectively. Again, significant differences were observed in the specific GPx-3 activity between healthy subjects and septic patients ($p = 0.005$) (Table 2).

The distribution patterns of serum GPx-3 bioactivity and GPx-3 protein concentration between normal subjects and septic patients were presented in Fig. 2. Although there was an overlap in lower ranges of GPx-3 bioactivity and GPx-3 protein concentration, it was noted that almost all septic patients had GPx-3 protein concentrations below 3.2 μg/ml except one 68-year-old female patient infected by *Klebsiella pneumoniae* with GPx-3 protein concentration at 6.56 μg/ml.

Further analysis of distribution of specified ranges of GPx-3 protein concentration and bioactivity were conducted in normal healthy subjects and septic patients (Fig. 2). The specified cut-off concentrations of GPx-3 protein and bioactivity were taken from the mean values in normal subjects (3.1 μg/ml) and septic patients (78.1 U/l), respectively. The results showed that a total of 88% normal subjects had GPx-3 bioactivity > 78.1 U/l, regardless of GPx-3 protein concentration (Table 3), indicating the importance of high GPx-3 bioactivity in maintaining normal body functions. Only about 12% of normal subjects had GPx-3 protein concentration < 3.1 μg/ml, and together with low GPx-3 bioactivity < 78.1 U/l.

A surprisingly high proportion of the septic patients (97%) with GPx-3 protein concentrations < 3.1 μg/ml, among them a half of septic patients had low GPx-3 bioactivity < 78.1 U/l. Only 3% of the septic patients had good GPx-3 protein concentrations (> 3.1 μg/ml) and GPx-3 bioactivity > 78.1 U/l. It was noted that none of the subjects from normal population or septic patients had high GPx-3 protein

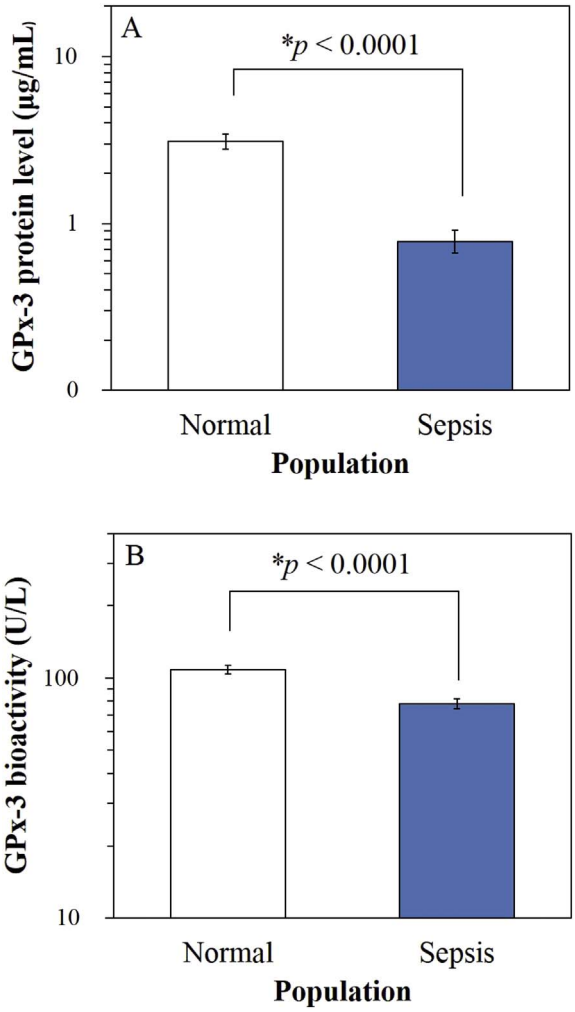

Fig. 1. Comparison of (A) GPx-3 bioactivity and (B) GPx-3 protein concentration in the serum samples from normal subjects and patients with sepsis. The graphs plot geometric means with the error bars of multiplying or dividing by one SEM. *$p < 0.0001$, Student's *t*-test.

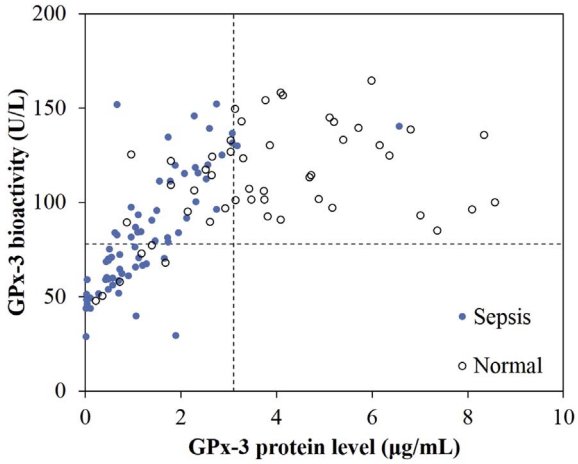

Fig. 2. Two-dimensional scatter plot to compare GPx-3 bioactivity with GPx-3 protein level in serum samples from normal subjects and patients with sepsis. The dash lines are representing the GPx-3 protein level at 3.1 μg/ml and GPx-3 bioactivity at 78.1 U/l.

Table 3
Distribution of specified GPx-3 protein concentration and GPx-3 bioactivity in normal subjects and patients with sepsis.

	% of selected population	
	Normal subjects	Patients with sepsis
GPx-3 protein concentration < 3.1 μg/ml		
With GPx-3 bioactivity < 78.1	12%	50%
With GPx-3 bioactivity > 78.1	26%	47%
GPx-3 protein concentration > 3.1 μg/ml		
With GPx-3 bioactivity < 78.1	0%	0%
With GPx-3 bioactivity > 78.1	62%	3%

Unit of GPx-3 bioactivity is U/l.

concentration but low GPx-3 bioactivity < 78.1 U/l (Table 3).

These results suggested that in patients with severe disease like sepsis, GPx-3 protein concentration may have gradually declined and become insufficient to sustain the required GPx-3 bioactivity for the normal body functions.

3.3. Correlation of GPx-3 bioactivity and GPx-3 protein concentration

A positive correlation between serum GPx-3 bioactivity and its corresponding protein concentration was observed in septic patients alone (sepsis alone, R = 0.74, $p < 0.001$; entire data set, R = 0.63, $p < 0.001$). However, the significance of the correlation between GPx-3 bioactivity and GPx-3 protein concentration was weaker among normal subjects (R = 0.41, $p = 0.004$) than patients with sepsis.

Simple linear regression model and multiple regression model were used to further investigate whether the GPx-3 bioactivity increases can be predicted by GPx-3 protein concentration, and to determine if there are other potential confounding factors. Covariates of gender and age included in the regression analysis were chosen based on the previously identified factors that have been shown to affect the GPx bioactivity in human [17–19].

In the normal subjects, a significant association was observed between GPx-3 bioactivity and gender ($p = 0.004$), but not with age (p = NS) (Table 4A). The association between GPx-3 bioactivity and gender remained significant after adjusting for GPx-3 protein concentration ($p = 0.026$) (Table 4B). A modest decrease of coefficient of GPx-3 protein concentration and statistically significant gender effect imply that gender is a modifier in the association between GPx-3 bioactivity and its protein concentration. The result was consistent with the previous report showing that GPx-3 bioactivity in female was higher than in male [17].

In order to investigate the impact of the 20 missing age values, we first compared the statistical results (Table 4B) of models 1 and 2 (n = 50) with models $1^{\#}$ and $2^{\#}$ (n = 30), respectively. Both comparisons show that the association between the GPx-3 bioactivity and protein concentration among those 30 normal subjects (with age data) is much stronger than the entire normal subjects. This indicates that the missing data of age were possible to be non-random. Therefore, which set of normal subjects' data is more appropriate to represent the normal population is unclear. We also use the model $3^{\#}$ (based on the same 30 subjects) to compare the models $1^{\#}$ and $2^{\#}$ (Table 4B) to investigate whether age plays an important role in the prediction of GPx-3 bioactivity. It appears that both age and gender do not play a major role in the change of GPx-3 bioactivity among these 30 normal subjects because the value of the R^2 are very similar for all 3 models.

In patients with sepsis, the regression results demonstrated that the association between GPx-3 bioactivity and GPx-3 protein concentration were significant even after adjusting for gender and age ($p < 0.0001$, Table 5A). The addition of covariates into the model slightly changed the regression coefficients (Table 5B), indicating a strong correlation (R > 0.7) between GPx-3 bioactivity and GPx-3 protein concentration in septic patients and not likely to be affected by gender and age. Compared to normal subjects, the impact of gender on GPx-3 activity in septic patients became negligible and the GPx-3 protein concentration likely played a major role in the change of GPx-3 bioactivity under the severe disease like sepsis.

4. Discussion

Although the human GPx-3 protein concentration and its bioactivity have been previously investigated in young healthy subjects and preeclamptic pregnant women [17,20], this is the first study to evaluate the human GPx-3 bioactivity and its associated GPx-3 protein concentration in serum samples collected from normal healthy subjects and patients with severe illness such as sepsis. Our major finding of this study is that the serum GPx-3 protein concentration and GPx-3 bioactivity were significantly lower in patients with sepsis than in normal subjects. The significant positive correlation between GPx-3 bioactivity and its protein concentration in septic patients appears to be independent from gender and age.

Due to the small sample size and unmatched ratios across races, the effect of race on GPx-3 bioactivity was not evaluated within the normal subject group or septic patient group by multiple linear regression model. The declined GPx-3 bioactivity observed in septic patients compared to normal subjects was unlikely to be caused by ethnic differences because it was reported that the GPx-3 bioactivity of African American was similar to Caucasian [19]. In addition, the age impact on the association with GPx-3 bioactivity in normal subjects cannot be confirmed due to large percentage of age information is missing. Further studies on age and race may be needed.

Until now, the underlying mechanism of the regulation of GPx-3 expression and its physiological and patho-physiological roles in sepsis remains limited. Up-regulation of GPx-3 protein is expected to scavenge the over-produced reactive oxygen species (ROS) when oxidative stress is present [10]. Whereas the acute oxidative stress seen in septic patients, the bioactivity of GPx-3 was found to be inversely associated with the disease severity [4]. In accordance with our study results, the decrease in GPx-3 bioactivity was suggested to be mainly attributed to the significant decline of GPx-3 protein concentration when patients suffering from severe disease like sepsis.

The likely explanation for this protein down-regulation is due to insufficient endogenous concentration of Se in septic patients. GPx-3 is primarily synthesized in kidney proximal tubules and actively secreted to plasma [21]. During synthesis, tissue form of Se is first converted to selenide as substrate for selenophosphate synthetase, and then selenophosphate is utilized to transform seryl-$tRNA^{ser}$ into selenocysteryl-$tRNA^{Sec}$. By decoding UGA codons in GPx-3 mRNA as selenocysteine, Se can be inserted co-translationally into the growing peptide chain of GPx-3 [22]. As a consequence, the synthesis of GPx-3 is expected to be responsive to Se availability and the regulation of GPx-3 expression by Se is mainly at the level of translation, though the mechanisms of mRNA reduction were also been reported in previous studies [23,24].

GPx enzymes have also been noted to be modified post-translationally such as acetylation or phosphorylation which could enhance the enzyme bioactivity [25]. Jacobson et al. reported markedly different 2D-PAGE results for the normal (high GPx-3 protein concentration) and disease (low GPx-3 protein concentration) plasma samples: over fifteen proteins with a wide range of pI and molecular weight values were detected from the high GPx-3 sample while only 3 proteins from the low GPx-3 sample [15]. It was suggested that the concentrations of detectable immunoreactive GPx-3 preserved in various states of synthesis were higher in normal plasma sample. Taken together with our results of distribution analysis (Table 3), the down-regulation of GPx-3 in septic patients may reduce the concentration of inactive GPx-3 and leave the intact one to some degree, which explains the elevation of specific GPx-3 activity and stronger correlation between GPx-3

Table 4
Association of GPx-3 bioactivity and GPx-3 protein concentration in the serum samples from normal subjects. (A) Simple linear regression model of GPx-3 bioactivity versus GPx-3 protein concentration and covariates of gender and age separately, (B) Multiple linear regression model of GPx-3 bioactivity versus GPx-3 protein concentration, sequentially adjusting for gender and age.

(A)

Covariates	Coefficient	95% CI	*p* value	Sample size (n)
GPx-3 protein concentration	5.36	1.85–8.87	0.004*	50
Gender[a]	21.76	7.11–36.41	0.004*	50
Age[b]	− 0.08	− 1.26–1.10	NS	30

(B)

Model	Covariates	Coefficient	95% CI	*p* value	Adjusted R^2	Sample size (n)
1	GPx-3 protein concentration	5.36	1.85–8.87	0.004*	0.15	50
2	GPx-3 protein concentration	4.20	0.68–7.71	0.020*	0.22	50
	Gender[a]	16.71	2.10–31.32	0.026*		
1[c]	GPx-3 protein concentration	11.48	7.08–15.88	< 0.0001*	0.49	30
2[c]	GPx-3 protein concentration	9.44	3.97–14.91	0.001*	0.50	30
	Gender[a]	12.64	− 7.75–33.03	NS		
3[c]	GPx-3 protein concentration	9.04	3.22–14.86	0.004*	0.48	30
	Gender[a]	15.16	− 8.27–38.57	NS		
	Age	0.22	− 0.73–1.17	NS		

CI, confidence interval.
* Significant difference ($p < 0.05$).
[a] Male = 0, female = 1.
[b] Data from 30 out of 50 subjects were used for analysis.
[c] Data from 30 out of 50 subjects were included in the models.

bioactivity and protein concentration compared to normal subjects.

The outcome of multiple organ dysfunction or failure could also result in reduction of GPx-3 expression, since GPx-3 is chiefly produced in kidney and also in other organs [21]. It was reported that not erythrocyte GPx-1 bioactivity but plasma GPx-3 bioactivity was reduced in patient with renal impairment [26]. Moreover, plasma GPx-3 bioactivity was found to be negatively correlated with both serum creatinine concentration and blood urea nitrogen [26], supporting that GPx-3 bioactivity could be largely affected by the renal function.

There are some limitations of this study. First, since the samples were collected from blood donors recruited by vendor, this may not be appropriate to represent the populations of interest. Second, due to large percentage of age and race information missing, the age and race impacts on the GPx-3 bioactivity could not be confirmed. Third, although a positive correlation was observed between GPx-3 bioactivity and its protein concentration, we could not determine how the GPx-3 bioactivity decreased with the GPx-3 protein concentration during the disease progression by this study. Last, the population of septic patients was not identified by severity of sepsis in this study, which would enlarge the variations in GPx-3 bioactivity and GPx-3 protein concentration of septic patients because the GPx-3 bioactivity was reported to be lower in patients with severe sepsis [4].

Sepsis is a leading cause of death in critically ill patients. Nowadays, a potential use of Se supplementation has been proposed to restore the

Table 5
Association of GPx-3 bioactivity and GPx-3 protein concentration in the serum samples from patients with sepsis. (A) Simple linear regression model of GPx-3 bioactivity versus GPx-3 protein concentration and covariates of gender and age separately (B) Multiple linear regression model of GPx-3 bioactivity versus GPx-3 protein concentration, sequentially adjusting for gender and age.

(A)

Covariates	Coefficient	95% CI	*p* value	Sample size (n)
GPx-3 protein concentration	21.31	16.66–25.97	< 0.0001*	70
Gender[a]	− 9.04	− 24.10–6.02	NS	70
Age	0.24	− 2.16–0.69	NS	70

(B)

Model	Covariates	Coefficient	95% CI	*p* value	Adjusted R^2	Sample size (n)
1	GPx-3 protein concentration	21.31	16.66–25.97	< 0.0001*	0.54	70
2	GPx-3 protein concentration	21.17	16.50–25.84	< 0.0001*	0.55	70
	Gender[a]	0.25	− 16.07–4.32	NS		
3	GPx-3 protein concentration	20.94	16.28–25.61	< 0.0001*	0.55	70
	Gender[a]	− 6.47	− 16.72–3.78	NS		
	Age	0.16	− 0.15–0.47	NS		

* Significant difference ($p < 0.05$).
[a] Male = 0, female = 1.

GPx-3 bioactivity to normal range and thus benefit the patients with sepsis [12–15], while two recent clinical trials did not conclude beneficial effects of Se supplementation to patients with severe sepsis in non-Se deficient populations [27,28]. In addition, selenite supplementation in patients with coronary artery disease has also proved to increase both GPx-1 protein and bioactivity in a dose-dependent manner [29]. Further understanding of the change of the biomarkers including GPx-3 activity and GPx-3 protein concentration by a longitudinal analysis could help discover the timing of the intervention of Se supplementation to interrupt the disease progression by increasing the GPx-3 protein level and its bioactivity, and therefore improve the overall re-dox state in patients with sepsis.

In conclusion, the GPx-3 protein concentration and GPx-3 bioactivity were both significantly declined in septic patients than in normal healthy subjects by 4.0- and 1.4-fold, respectively. A positive correlation between GPx-3 bioactivity and GPx-3 protein concentration was established by a cross-sectional regression. These findings suggest that the drop in GPx-3 bioactivity in septic patients was mainly attributed to the reduced GPx-3 protein concentration.

Acknowledgements

We acknowledge XenoBiotic Laboratories, Inc. for technical support. We gratefully thank Dr. Jain Chung for providing valuable insights and analysis support of statistical content. All authors have read the journal's policy on conflicts of interest and authorship agreement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2017.10.031.

References

[1] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G.R. Bernard, J. Chiche, C.M. Coopersmith, R.S. Hotchkiss, M.M. Levy, J.C. Marshall, G.S. Martin, S.M. Opal, G.D. Rubenfeld, T. Poll, J. Vincent, D.C. Angus, The third international consensus definitions for sepsis and septic shock (sepsis-3), JAMA 315 (8) (2016) 801–810.

[2] J. Duran-Bedolla, M.A.M. de Oca-Sandoval, V. Saldaña-Navor, J.A. Villalobos-Silva, M.C. Rodriguez, S. Rivas-Arancibia, Sepsis, mitochondrial failure and multiple organ dysfunction, Clin. Invest. Med. 37 (2) (2014) 58–69.

[3] S.L. Fan, N.S. Miller, J. Lee, D.G. Remick, Diagnosing sepsis–the role of laboratory medicine, Clin. Chim. Acta 460 (2016) 203–210.

[4] W. Manzanares, A. Biestro, F. Galusso, M.H. Torre, N. Mañay, G. Pittini, G. Facchin, G. Hardy, Serum selenium and glutathione peroxidase-3 activity: biomarkers of systemic inflammation in the critically ill? Intensive Care Med. 35 (5) (2009) 882–889.

[5] L.V. Papp, J. Lu, A. Holmgren, K.K. Khanna, From selenium to selenoproteins: synthesis, identity, and their role in human health, Antioxid. Redox Signal. 9 (7) (2007) 775–806.

[6] V. Mishra, M. Baines, S.E. Perry, P.J. McLaughlin, J. Carson, R. Wenstone, A. Shenkin, Effect of selenium supplementation on biochemical markers and outcome in critically ill patients, Clin. Nutr. 26 (1) (2007) 41–50.

[7] W. Manzanares, A. Biestro, F. Galusso, M.H. Torre, N. Mañáy, G. Facchin, G. Hardy, High-dose selenium for critically ill patients with systemic inflammation: pharmacokinetics and pharmacodynamics of selenious acid: a pilot study, Nutrition 26 (6) (2010) 634–640.

[8] W. Manzanares, A. Biestro, M.H. Torre, F. Galusso, G. Facchin, G. Hardy, High-dose selenium reduces ventilator-associated pneumonia and illness severity in critically ill patients with systemic inflammation, Intensive Care Med. 37 (7) (2011) 1120–1127.

[9] V. Janka, K. Ladislav, Restoration of antioxidant enzymes in the therapeutic use of selenium in septic patients, Wien. Klin. Wochenschr. 125 (11 – 12) (2013) 316–325.

[10] Y. Miyamoto, Y.H. Koh, Y.S. Park, N. Fujiwara, H. Sakiyama, Y. Misonou, T. Ookawara, K. Suzuki, K. Honke, N. Taniguchi, Oxidative stress caused by inactivation of glutathione peroxidase and adaptive responses, Biol. Chem. 384 (4) (2003) 567–574.

[11] M.W. Angstwurm, J. Schottdorf, J. Schopohl, R. Gaertner, Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome, Crit. Care Med. 27 (9) (1999) 1807–1813.

[12] M.W. Angstwurm, L. Engelmann, T. Zimmermann, C. Lehmann, C.H. Spes, P. Abel, R. Strauss, A. Meier-Hellmann, R. Insel, J. Radke, J. Schüttler, R. Gärtner, Selenium in intensive care (SIC): results of a prospective randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock, Crit. Care Med. 35 (1) (2007) 118–126.

[13] J. Valenta, H. Brodska, T. Drabek, J. Hendl, A. Kazda, High-dose selenium substitution in sepsis: a prospective randomized clinical trial, Intensive Care Med. 37 (5) (2011) 808–815.

[14] G. Woth, B. Nagy, Á. Mérei, B. Ernyey, R. Vincze, Z. Kaurics, J. Lantos, L. Bogár, D. Mühl, The effect of Na-selenite treatment on the oxidative stress–antioxidants balance of multiple organ failure, J. Crit. Care 29 (5) (2014) 883.e7–883.e11.

[15] G.A. Jacobson, C. Narkowicz, Y.C. Tong, G.M. Peterson, Plasma glutathione peroxidase by ELISA and relationship to selenium level, Clin. Chim. Acta 369 (1) (2006) 100–103.

[16] M.M. Levy, M.P. Fink, J.C. Marshall, E. Abraham, D. Angus, D. Cook, J. Cohen, S.M. Opal, J. Vincent, G. Ramsay, 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference, Intensive Care Med. 29 (4) (2003) 530–538.

[17] J.W. Rush, S.D. Sandiford, Plasma glutathione peroxidase in healthy young adults: influence of gender and physical activity, Clin. Biochem. 36 (5) (2003) 345–351.

[18] S.E. Espinoza, H. Guo, N. Fedarko, A. DeZern, L.P. Fried, Q.L. Xue, S. Leng, B. Beamer, J.D. Walston, Glutathione peroxidase enzyme activity in aging, J. Gerontol. A Biol. Sci. Med. Sci. 63 (5) (2008) 505–509.

[19] M.K. Hargreaves, J. Liu, M.S. Buchowski, K.A. Patel, C.O. Larson, D.G. Schlundt, D.M. Kenerson, K.E. Hill, R.F. Burk, W.J. Blot, Plasma selenium biomarkers in low income black and white Americans from the southeastern United States, PLoS One 9 (1) (2014) e84972.

[20] H.D. Mistry, V. Wilson, M.M. Ramsay, M.E. Symonds, F.B. Pipkin, Reduced selenium concentrations and glutathione peroxidase activity in preeclamptic pregnancies, Hypertension 52 (5) (2008) 881–888.

[21] N. Avissar, D.B. Ornt, Y. Yagil, S. Horowitz, R.H. Watkins, E.A. Kerl, K. Takahashi, I.S. Palmer, H.J. Cohen, Human kidney proximal tubules are the main source of plasma glutathione peroxidase, Am. J. Phys. 266 (1994) C367–375.

[22] B.J. Lee, P.J. Worland, J.N. Davis, T.C. Stadtman, D.L. Hatfield, Identification of a selenocysteyl-tRNA(Ser) in mammalian cells that recognizes the nonsense codon, UGA, J. Biol. Chem. 264 (17) (1989) 9724–9727.

[23] N. Avissar, E.A. Kerl, S.S. Baker, H.J. Cohen, Extracellular glutathione peroxidase mRNA and protein in human cell lines, Arch. Biochem. Biophys. 309 (2) (1994) 239–246.

[24] P.M. Moriarty, C.C. Reddy, L.E. Maquat, Selenium deficiency reduces the abundance of mRNA for se-dependent glutathione peroxidase 1 by a UGA-dependent mechanism likely to be nonsense codon-mediated decay of cytoplasmic mRNA, Mol. Cell. Biol. 18 (5) (1998) 2932–2939.

[25] E.N. Reinke, D.N. Ekoue, S. Bera, N. Mahmud, A.M. Diamond, Translational regulation of GPx-1 and GPx-4 by the mTOR pathway, PLoS One 9 (4) (2014) e93472.

[26] A. Mohamed, M.A. Bakr, S.E. Farahat, E.A.A. El-Fattah, Glutathione peroxidase activity in patients with renal disorders, Clin. Exp. Nephrol. 9 (2) (2005) 127–131.

[27] Y. Sakr, V.P. Maia, C. Santos, J. Stracke, M. Zeidan, O. Bayer, K. Reinhart, Adjuvant selenium supplementation in the form of sodium selenite in postoperative critically ill patients with severe sepsis, Crit. Care 18 (2) (2014) R68.

[28] F. Bloos, E. Trips, A. Nierhaus, J. Briegel, D.K. Heyland, U. Jaschinski, U. Jaschinski, O. Moerer, A. Weyland, G. Marx, M. Gründling, S. Kluge, I. Kaufmann, K. Ott, M. Quintel, F. Jelschen, P. Meybohm, S. Rademacher, A. Meier-Hellmann, S. Utzolino, U.X. Kaisers, C. Putensen, G. Elke, M. Ragaller, H. Gerlach, K. Ludewig, M. Kiehntopf, H. Bogatsch, C. Engel, F.M. Brunkhorst, M. Loeffler, K. Reinhart, for SepNet Critical Care Trials Group, Effect of sodium selenite administration and procalcitonin-guided therapy on mortality in patients with severe sepsis or septic shock: a randomized clinical trial, JAMA Intern. Med. 176 (9) (2016) 1266–1276.

[29] R. Schnabel, E. Lubos, C.M. Messow, C.R. Sinning, T. Zeller, P.S. Wild, D. Peetz, D.E. Handy, T. Munzel, J. Loscalzo, K.J. Lackner, S. Blankenberg, Selenium supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease: the SElenium therapy in coronary artery disease patients (SETCAP) study, Am. Heart J. 156 (6) (2008) (1201-e1).