OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2024.60.66

Research Article
Correlation Between GPx and Nrf2 Antioxidant Levels and Clinical Improvement in Ischemic Stroke Patients Receiving Glutathione Supplementation

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Abstract

Background and Objective: Acute ischemic stroke induces metabolic and biomolecular changes, resulting in brain functional disorders and morphological damage as well as increased production of free radicals and reactive oxygen species (ROS). The antioxidant defense system in stroke includes glutathione peroxidase (GPx) and nuclear erythroid-related factor 2 (Nrf2), with glutathione playing a vital role in reducing ROS levels. This study aimed to assess the correlation between serum GPx and Nrf2 levels and clinical improvement in acute ischemic stroke patients receiving glutathione supplementation. **Materials and Methods:** A randomized controlled trial was conducted at Dr. Kariadi Hospital, Semarang, from September 2021 to January 2022, with acute ischemic stroke patients diagnosed through head CT as the study population. Serum GPx and Nrf2 levels were collected within $\leq$72 hrs of stroke onset and clinical outcomes were evaluated using National Institutes of Health Stroke Scale (NIHSS). Data normality was examined using Kolmogorov-Smirnov test. Correlation and association were tested using Spearman correlation and Mann-Whitney U test, respectively. Statistical significance was set at $p<0.05$. **Results:** From a total of 40 subjects, serum GPx and Nrf2 levels were significantly elevated in the treatment group compared to controls ($p = 0.000$ and $p = 0.030$, respectively). A significant positive correlation was observed between elevated serum GPx levels and clinical improvement ($r = 0.464$, $p = 0.039$), while no significant correlation was found between Nrf2 levels and clinical improvement ($r = -0.033$, $p = 0.889$). **Conclusion:** In conclusion, there was a positive correlation between elevated serum GPx levels and clinical improvement in acute ischemic stroke patients receiving glutathione supplementation. The same could not be said regarding Nrf2 levels. These findings emphasize the potential of GPx as a therapeutic target in stroke management.

Key words: Glutathione peroxidase (GPx), ischemic stroke, National Institutes of Health Stroke Scale (NIHSS). nuclear erythroid-related factor 2 (Nrf2)

Citation: Retnaningsih, D. Tugasworo, H. Suryawati, M.I. Widiastuti and D. Pudjonarko, *et al.*,, 2024. Correlation between GPx and Nrf2 antioxidant levels and clinical improvement in ischemic stroke patients receiving glutathione supplementation. Pak. J. Nutr., 23: 60-66.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acute ischemic stroke induces metabolic and molecular changes leading to brain damage and increased production of reactive oxygen species (ROS), exacerbating tissue injury. Oxidative stress, implicated in calcium-mediated nerve cell damage, also contributes to thrombus formation and atherosclerosis progression[1]. Endogenous antioxidants like glutathione peroxidase (GPx) and nuclear erythroid-related factor 2 (Nrf2) play crucial roles in mitigating oxidative stress through various mechanisms, such as reducing oxidized low-density lipoprotein (LDL) and improving endothelial function[2-6]. While studies have examined serum levels of GPx and Nrf2 in acute ischemic stroke patients, their correlation with neurological improvement after glutathione supplementation remains unexplored. This study aimed to investigate this relationship, shedding light on the potential therapeutic benefits of glutathione supplementation.

MATERIALS AND METHODS

This randomized controlled trial was conducted at Dr. Kariadi Hospital, Semarang, Indonesia, involving acute ischemic stroke patients whose onset was $\leq$72 hrs as the study population. The inclusion criteria were patients without inflammatory disorders, degenerative disorders, diabetes mellitus, or other malignancies, who provided written informed consent. Serum samples were obtained within 72 hrs of symptoms onset and head CT scans were performed to exclude intracranial hemorrhage. Neurological status was assessed using the NIHSS as a measure of improvement. The age threshold for being considered elderly is >60 years. Subjects are categorized as obese if their BMI is >30 kg/m^2, as having hypertension if their blood pressure is $\geq$140/90 mmHg and as having high LDL if their LDL level is higher than 100 mg/dL.

This self-funded study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital (Ethical Clearance Number 825/EC/KEPK-RSDK/2021, dated May 20, 2021). Oral supplementation with 500 mg Glutathione capsule every 12 hrs (at 7 AM and 7 PM) was administered for 14 days and monitored for side effects.

SPSS version 26 Statistical Software Program (SPSS, Inc., IBM, Chicago, Illinois, USA) for Windows was used for data management and analysis. Characteristics of the studied population was described in frequencies and percentages for nominal variables and mean or median for numeric variables. The Spearman correlation coefficient and Mann-Whitney U test were employed for analytical statistics, considering a $p<0.05$ as having statistical significance.

RESULTS

Recruitment of study participants: Figure 1 shows that our initial study population was 55 patients, all of whom had acute ischemic stroke. Of these, 13 were excluded due to not meeting the inclusion criteria and 2 refused to participate, resulting in 40 patients being randomized into the treatment and control groups.

Descriptive data: Table 1 shows the study participants' demographic characteristics. Forty study participants, comprising 25 men (62.5%) and 15 women (37.5%), were divided into control and treatment groups. The independent variables investigated in this study included serum levels of nuclear erythroid-related factor 2 (Nrf2) and glutathione peroxidase (GPx), body mass index (BMI), hypertension and serum level of low-density lipoprotein (LDL). Twenty four subjects (60%) were over 60 years old, with a median age of 62 (min 35, max 79) years old. Statistical analysis revealed no significant differences in demographic characteristics between the treatment and control groups.

The majority of ischemic stroke patients were non-obese (n = 36, 90%), had hypertension (n = 29, 72.5%) and exhibited high LDL levels (n = 28, 67.5%). The median LDL level was 127 mg/dL (min 60 mg/dL, max 250 mg/dL) and the median BMI was 25.35 kg/m^2 (min 19.5 kg/m^2, max 32.03 kg/m^2). On day 1, 35 subjects (87.5%) had moderate NIHSS score (6-14) while 5 subjects (12.5%) had severe NIHSS score (15-24).

Outcome data: Table 2 shows that on day 1, the mean serum GPx levels were lower in the treatment group (19.55±7.59) compared to the control group (21.35±6.31), with a significant difference ($p<0.027$). By day 14, the mean GPx levels increased significantly ($p<0.012$) in the treatment group (27.56±14.65) compared to the control group (20.26±3.12), resulting in a significantly ($p<0.000$) higher mean increase in GPx levels in the treatment group (8.02±9.357) compared to the control group (-1.09±6.58).

Similarly, on day 1, the mean Nrf2 levels of the treatment group (10.09±6.77) and the control group (10.10±5.52) were comparable, albeit with no significant difference ($p<0.267$). By day 14, although the mean Nrf2 levels were higher in the treatment group (14.32±11.16) compared to the control group (10.09±6.77), the difference was not statistically

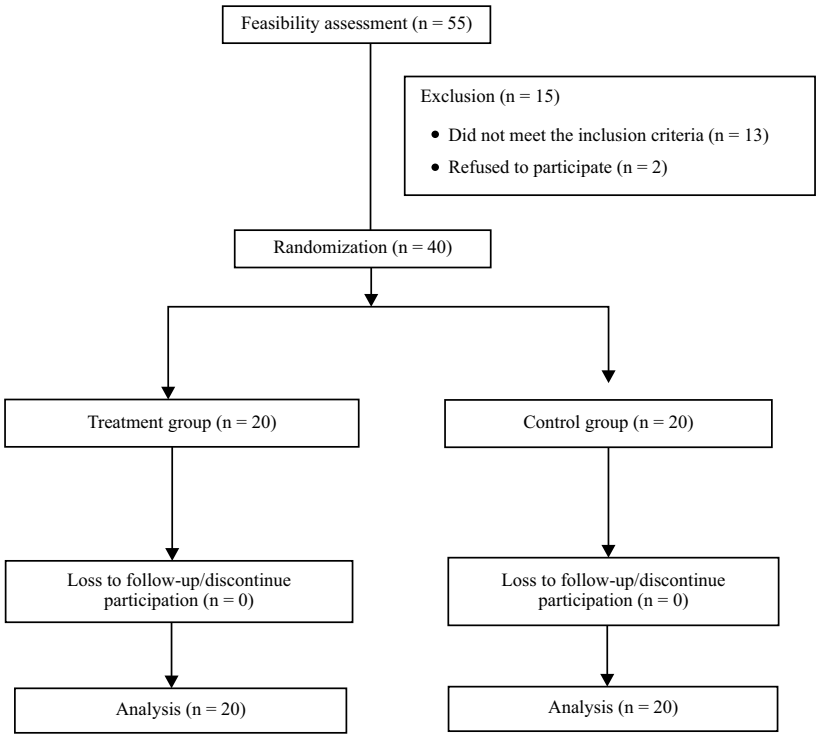

Fig. 1: Patient enrollment and allocation flowchart (according to the CONSORT guidelines)
*Spearman, rho = 0.021, p = 0.928 and *rho = 0.114, p = 0.632

Table 1: Demographic characteristics of study participants

	Treatment group		Control group		
Variables	No.	Percentage	No.	Percentage	p-value
Age					
>60 years	13	65	11	55	0.75
≤60 years	7	35	9	45	
Sex					
Male	12	60	13	65	0.52
Female	8	40	7	35	
Body mass index BMI					
Obese	3	5	1	15	0.30
Non-obese	17	95	19	85	
Blood pressure					
Hypertension	14	70	15	75	0.73
No hypertension	6	30	5	25	
Low-density lipoprotein LDL					
High >100	12	60	15	75	0.26
Normal ≤100	8	40	5	25	
NIHSS score					
Moderate 6-14	16	80	19	95	0.16
Severe 15-24)	4	20	1	5	

significant (p<0.064). However, the mean increase in serum Nrf2 levels was significantly (p<0.030) higher in the treatment group (4.22±9.12) compared to the control group (-0.97±5.78).

Table 3 shows that on day 1, the mean NIHSS score in the treatment group (11.05±2.66) was slightly higher than the control group (9.65±2.66), however, the difference was not statistically significant (p<0.117). By day 14, although the

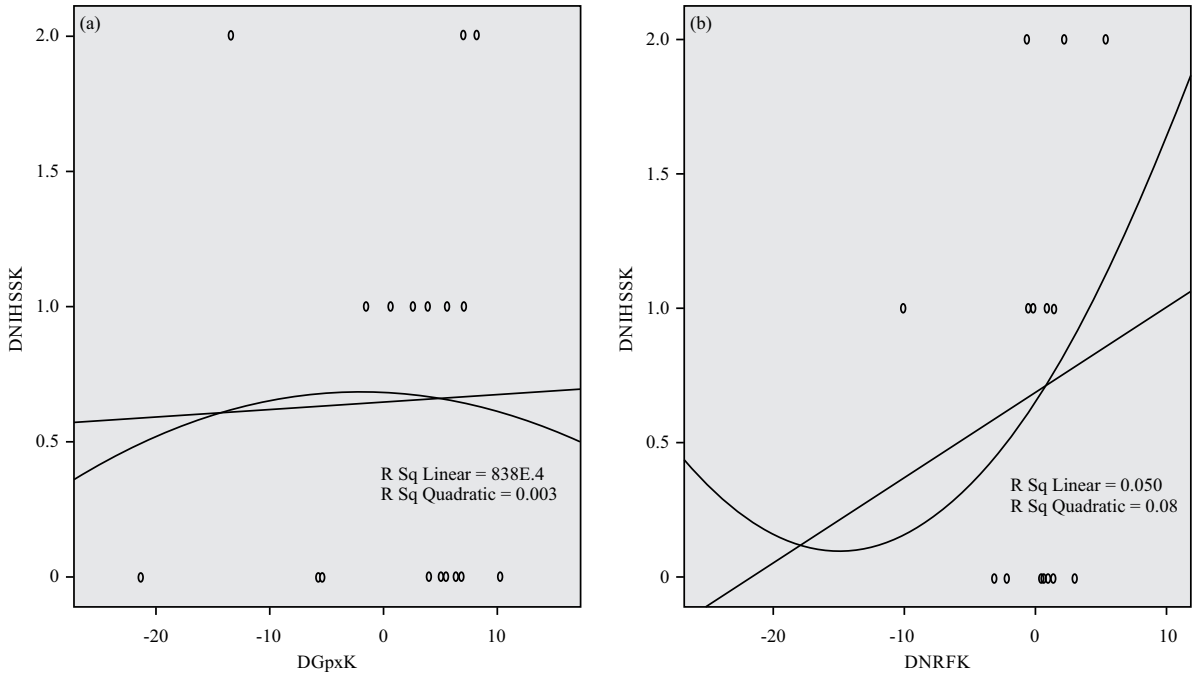

Fig. 2: Correlation between increased serum GPx and Nrf2 levels and clinical improvement in the control group
(a) rho = 0.464, p = 0.039; (b) rho = -0.033, p = 0.889

Table 2: Mean and median of serum GPx and Nrf2 levels in patients with acute ischemic stroke

	Treatment group		Control group		
Variables	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	p-value
GPx-1	19.55±7.59	16.92 (13-43)	21.35±6.31	19.88 (15-42)	0.027*
GPx-14	27.56±14.65	22.42 (17-80)	20.26±3.12	19.88 (15-42)	0.012*
Increase in GPx	8.02±9.357	5.88 (2-46)	-1.09±6.58	1.19 (-21-6)	0.000*
Nrf2-1	10.09±6.77	7.72 (3.65-30.9)	10.10± 5.52	8.80 (5.97-30.59)	0.267*
Nrf2-14	14.32±11.16	9.20 (7.33-43.66)	10.09±6.77	7.72 (3.65-30.90)	0.064*
Increase in Nrf2	4.22±9.12	1.54 (-13.30-24.95)	-0.97±5.78	0.41 (-21.95-5.36)	0.030*

*Mann-Whitney, p<0.05, Min: Minimum and Max: Maximum

Table 3: Mean and median of NIHSS score in patients with acute ischemic stroke

	Treatment group		Control group		
Variables	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)	p-value
NIHSS-1	11.05±2.66	11 (8-16)	9.65±2.66	9 (6-16)	0.117*
NIHSS-14	9.90±2.78	9.5 (6-16)	9.00±2.63	9 (5-15)	0.311*
Decrease in NIHSS	0.95±0.887	1 (0-2)	0.65±0.745	0.5 (0-2)	0.282*

*Mann-Whitney, p<0.05, Min: Minimum and Max: Maximum

average NIHSS score in the treatment group (9.9±2.78) was slightly higher than the control group (9±2.63), the difference remained statistically insignificant (p<0.311). Similarly, the mean increase in NIHSS score was slightly higher in the treatment group (0.95±0.887) compared to the control group (0.65±0.745) but again, the difference was not statistically significant (p<0.282).

No correlation was observed in this study between elevated serum GPx levels and NIHSS score (Spearman correlation coefficient = 0.021, p = 0.928) in the control group. The lack of correlation was also observed between serum Nrf2 levels and NIHSS score (Spearman correlation coefficient = 0.114, p<0.632) (Fig. 2).

This study also identified a positive correlation between elevated serum GPx levels and NIHSS score (Spearman correlation coefficient = 0.464, p = 0.039) in the treatment group. However, there was no correlation between elevated

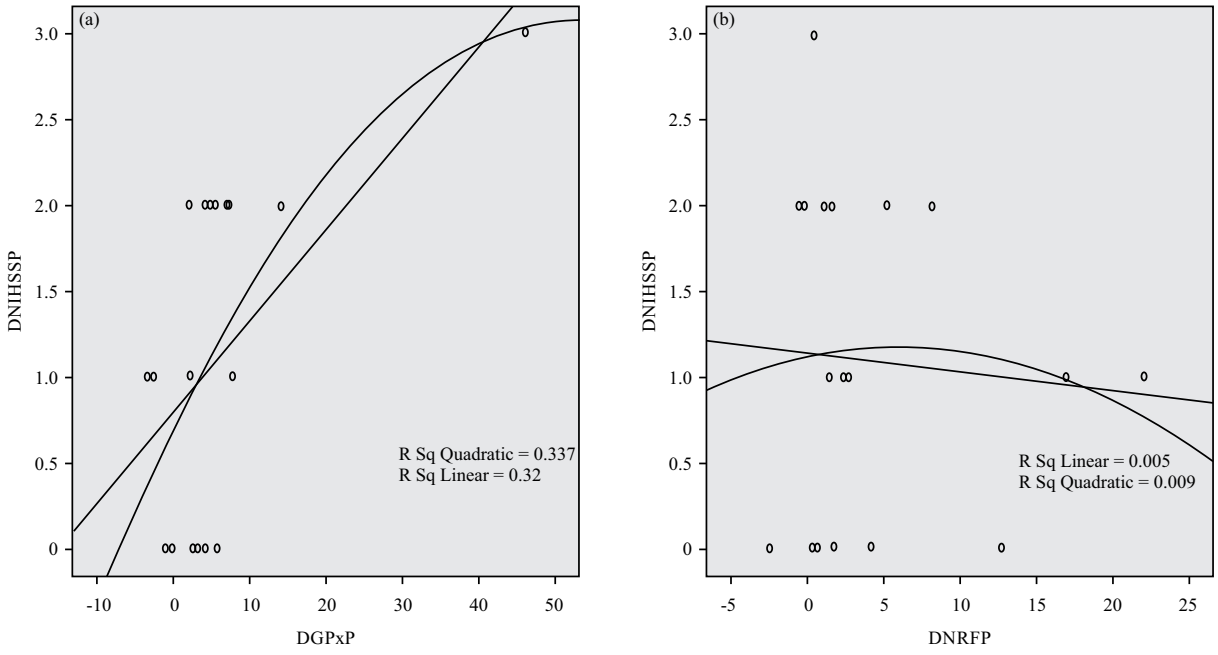

Fig. 3: Correlation between increased serum GPx and Nrf2 levels and clinical improvement in the treatment group
(a) rho = 0.464, p = 0.039; (b) rho = -0.033, p = 0.889

serum Nrf2 levels and clinical improvement, as assessed using NIHSS (Spearman correlation coefficient = -0.033, p = 0.889) (Fig. 3).

DISCUSSION

The majority of the 40 subjects included in our study were male (n = 25). This finding is consistent with a previous study conducted by Wafa *et al.*[7], who reported a higher frequency of ischemic stroke in men. Additionally, the majority of our subjects were over 60 years old, aligning with the average age reported in a previous study by O'Donnell *et al.*[8], where patients with ischemic stroke had an average age of 62.2 years. The increased frequency of ischemic stroke with advancing age can be attributed to the aging process, which contributes to mild inflammation and oxidative stress. This process resulted in thickening and reduced elasticity of the blood vessels, particularly the endothelium of the inner layer. Consequently, this process results in narrowing and collapsing of the blood vessel lumen, thereby reducing blood flow to the brain[7,8].

Our study observed a lower proportion of obese subjects, which contrasts with the findings of Chaudhary *et al.*[9] involving 2,767 (41.3%) obese patients. We also found a higher prevalence of hypertension among our subjects, consistent with the study conducted by Shaafi *et al.*[10], which identified hypertension as a significant risk factor for stroke. It is well-established that hypertension is the most important modifiable risk factor for stroke, exhibiting a strong, direct, linear and continuous association. Furthermore, elevated systolic blood pressure levels following a chronic stroke are associated with unfavorable neurological outcomes[9-11].

In our study, a higher proportion of subjects were found to have elevated LDL levels, consistent with the findings of Kartikasari *et al.*[12], in which 88.9% of patients with acute ischemic stroke exhibited higher LDL levels. Elevated levels of modified oxidized low-density lipoprotein (Ox-LDL), influenced by oxidative stress, play a crucial role in vascular endothelial lesions, inflammatory factor release, foam cell formation, plaque instability and increased thrombosis. Ox-LDL is implicated in both the initiation and acceleration of atherosclerosis.

According to the 2018 ACC/AHA Cholesterol Guidelines, patients with acute ischemic stroke are recommended to focus on lifestyle and diet modifications, along with medication for the treatment of dyslipidemia. Patients receiving statins can aim for optimal therapy with target LDL levels of 70 mg/dL or higher ($\geq$1.8 mmol/L), as supported by class I recommendation and level of evidence A[12-14].

Current study revealed a significantly elevated serum GPx levels in the treatment group. This finding is consistent with a previous study conducted by Sabetghadam *et al.*[15], who reported elevated serum GPx levels after 3 days of N-acetylcysteine (NAC) administration in acute ischemic stroke patients. Similarly, we observed a significant increase in serum Nrf2 levels in the treatment group. This finding is consistent with a previous study conducted by Song *et al.*[16] who reported a significant difference in Nrf2 levels in ischemic stroke patients.

Results of the present study revealed a significant correlation between elevated serum GPx levels and neurological improvement among acute ischemic stroke patients. A previous study by Sabetghadam *et al.*[15] reported similar results, indicating that exogenous antioxidant supplements, such as glutathione, can protect brain tissues against oxidative stress damage. Furthermore, our study suggested that early administration of glutathione, as early as within 24 hrs after the onset of acute ischemic stroke, is associated with better clinical outcomes. Specifically, we observed a higher proportion of patients experiencing improvement in the moderate NIHSS group, particularly in awareness and motor skills.

In the present study, correlation between increased serum Nrf2 levels and clinical improvement in acute ischemic stroke patients was not observed. Results of this study are different from those of Zhang *et al.*[17] who involved 127 acute ischemic stroke patients, there was a significant difference in serum Nrf2 levels between the treatment and control groups following antioxidant administration for 2 weeks ($p<0.05$). The study also investigated other variables associated with the Keap1-Nrf2/ARE signaling pathway, such as kelch-like erythroid cell-derived protein 1 (Keap1) levels and antioxidant response element (ARE) activity. Activation of the Keap1-Nrf2/ARE pathway has been shown to have anti-inflammatory, antioxidant and anti-apoptotic effects following ischemic stroke through the upregulation of downstream factors such as heme oxygenase 1 (HO-1), NADPH quinone oxidoreductase (NQO1) and glutathione peroxidase (GPx)[17-19].

However, it is worth noting that our study did not measure serum Keap1 and ARE levels, which may influence Nrf2 levels and NIHSS scores. Further studies considering these factors could provide a more comprehensive understanding of the role of the Keap1-Nrf2/ARE pathway in acute ischemic stroke.

CONCLUSION

Our study found a significant positive correlation between increased serum glutathione peroxidase (GPx) levels and clinical improvement in acute ischemic stroke patients receiving glutathione supplementation. This suggests that higher GPx levels may be associated with better clinical outcomes in this population. In contrast, we did not observe a significant association between increased serum Nrf2 levels and clinical improvement in patients receiving supplementation. This indicates that Nrf2 levels may not be a reliable predictor for the clinical outcomes of acute ischemic stroke patients receiving glutathione supplementation. Overall, our findings highlight the potential importance of GPx levels as a biomarker for monitoring and predicting clinical improvement in acute ischemic stroke patients receiving glutathione supplementation. Further research is needed to elucidate the underlying mechanisms and validate these findings in larger patient cohorts.

ACKNOWLEDGMENTS

The authors would like to thank the doctors and nurses of Dr. Kariadi Hospital, Semarang, Indonesia, for providing technical assistance during the conduct of this study.

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