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A Preliminary Study on the Potential Protective Role of the Antioxidative Stress Markers of Cognitive Impairment: Glutathione and Glutathione Reductase

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Objective: To investigate the relationship between reduced glutathione (GSH), a key molecule of the antioxidant defense system in the blood, and glutathione reductase (GR), which reduces oxidized glutathione (glutathione disulfide [GSSG]) to GSH and maintains the redox balance, with the prevalence of Alzheimer's dementia and cognitive decline.
Methods: In all, 20 participants with Alzheimer's dementia who completed the third follow-up clinical evaluation over 6 years were selected, and 20 participants with normal cognition were selected after age and sex matching. The GSH and GR concentrations were the independent variables. Clinical diagnosis and neurocognitive test scores were the dependent variables indicating cognitive status.

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Results: The higher the level of GR, the greater the possibility of having normal cognition than of developing Alzheimer's dementia. Additionally, the higher the level of GR, the higher the neurocognitive test scores. However, this association was not significant for GSH. After 6 years, the conversion rate from normal cognition to cognitive impairment was significantly higher in the lower 50th percentile of the GR group than in the upper 50th percentile.
Conclusion: The higher the GR, the lower the prevalence of Alzheimer's dementia and incidence of cognitive impairment and the higher the cognitive test scores. Therefore, GR is a potential protective biomarker against Alzheimer's dementia and cognitive decline.

KEY WORDS: Alzheimer dementia; Oxidative stress; Biomarker.

INTRODUCTION

Alzheimer's dementia is a chronic neurodegenerative disease and the most common cause of dementia [1]. Many pathological changes occur before Alzheimer's dementia is clinically diagnosed, followed by cognitive decline and deterioration of activities of daily life [2,3]. Therefore, the diagnosis of Alzheimer's dementia in the prodromal phase, before the clinical symptoms appear, plays an important role in delaying or stopping the course of the disease through early intervention [4]. Oxidative stress plays an important role in the pathology of Alzheimer's dementia, especially in the early course, and precedes the hallmarks of Alzheimer's dementia, such as neurofibrillary tangle (tau) and senile plaque (beta amyloid) [5,6].

Oxidative stress occurs when too many reactive oxygen species are produced, damaging the structure and function of brain cells; antioxidant systems protect against this damage. When these systems function properly, the oxidation-reduction balance is maintained. In the early stage of oxidation-reduction imbalance, free radicals cause oxidative stress, damage, inflammation, and cell death. Harmful oxygen species mainly originate during the generation of superoxide free radicals, and the accumulation of H_2O_2 generates a large amount of reactive hydroxyl free radicals [7]. The antioxidant systems play a role in scavenging these harmful superoxide formations. Among them, the glutathione system, including both the reduced (GSH) and oxidized (glutathione disulfide [GSSG]) forms, is attracting attention. GSH is the substrate for glutathione peroxidase (removing hydroxyl free radicals) [8], and GSSG is converted back to GSH by glutathione reductase (GR) (Fig. 1). Therefore, GSH and GR are the antioxidants that play a major role in maintaining the redox balance in the human body.

A previous meta-analysis study has implicated oxidative stress in Alzheimer's disease and mild cognitive impairment (MCI). The authors observed an intracellular GSH decrease in MCI and both intra- and extracellular decreases in AD and suggested that changes in glutathione

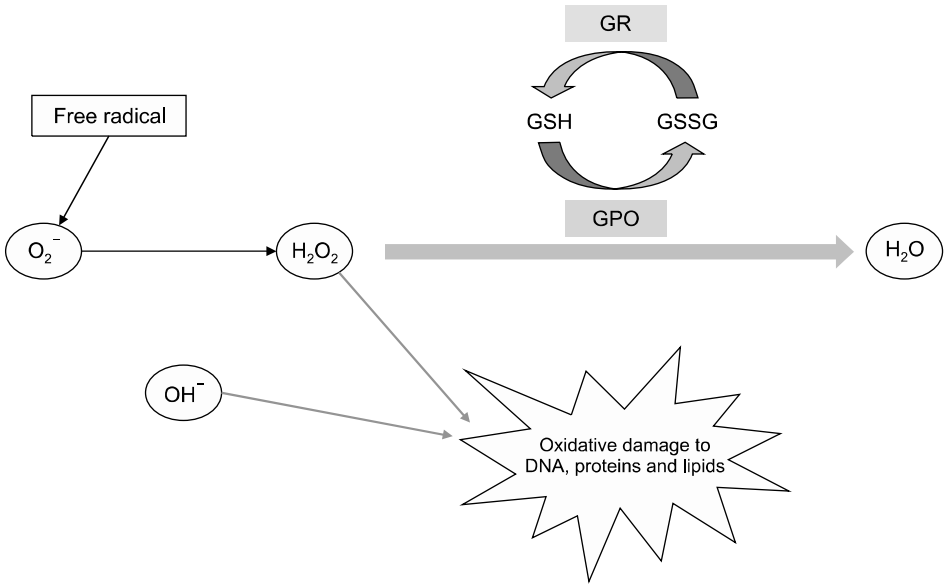

Fig. 1. Antioxidant systems.
GSH, glutathione; GSSG, oxidized form of GSH; GR, glutathione reductase; SOD, superoxide dismutase; GPO, glutathione peroxidase. Free radicals generated in various pathogenic processes can cause oxidative damage, and our body reduces them through antioxidant systems such as SOD, GSH, and GPO.

levels are associated with the occurrence of Alzheimer's disease and mild cognitive impairment [9]. Given the etiological importance of oxidative stress in Alzheimer's dementia, several studies have measured the glutathione system using blood samples of patients with Alzheimer's dementia. Previous studies have attempted to confirm whether there is a statistically significant difference between glutathione (GSH and GSSG), glutathione peroxidase, and GR levels through comparisons of groups with normal cognition and Alzheimer's dementia (or MCI), but the results have been controversial [5,10-13]. Previous studies have shown that GR and GSH levels are lower in patients with Alzheimer's dementia than in those without this condition [5]; however, some studies showed no significant differences in GSH levels between the groups [13]. Moreover, there are very few long-term studies on the effect of these blood markers on cognitive function [10]. Furthermore, to our knowledge, no study has simultaneously measured these blood markers, especially GR and GSH, in the same participants.

Therefore, we attempted to determine whether baseline GSH and GR levels measured from plasma samples of participants with normal cognition and Alzheimer's dementia are related to their current cognitive function and whether they are associated with cognitive decline over a given period of time.

METHODS

Study Sample

This study was conducted as part of a prospective study on cognitive aging and dementia in Koreans (Korean Longitudinal Study on Cognitive Aging and Dementia, KLOSCAD). The KLOSCAD is a large-scale community multi-institution cohort conducted to develop standard dementia prevention and management guidelines and models for Koreans [14]. A total of 6,818 people participated in the baseline survey from November 2010 to October 2012. The KLOSCAD cohort was followed every 2 years, and the fourth follow-up was completed as of March 2022. The KLOSCAD was approved by the Institutional Review Board of Seoul National University Bundang Hospital (No. B-0912/089-010). Before enrollment to the study, all participants voluntarily signed a consent form after receiving an explanation of the purpose and method of the study. This study included 20 participants with Alzheimer's dementia whose baseline blood samples were collected and who had completed all evaluations including clinical diagnosis during the third follow-up (6 years) and a neuropsychological examination during the second follow-up (4 years); 20 participants with normal cognition and who met the abovementioned conditions were randomly sampled by age and sex matching. We randomly selected 20 Alzheimer's dementia patients using a random number generator program available on Research Randomizer2 (https://www.randomizer.org/). We considered the cost-benefit and accordingly conducted the study within the budget. This research is a preliminary study; if good results are obtained, larger studies will be conducted accordingly.

Measurement

Clinical diagnosis

To diagnose cognitive disorders, geriatric neuropsychiatrists specializing in dementia research conducted a face-to-face standardized diagnostic interview including physical and neurologic examinations using the Consortium to Establish a Registry for Alzheimer's Disease Assessment Packet (CERAD-K) clinical assessment battery [15]. Using the principle of the Clinical Dementia Rating® scale, the severity of dementia was evaluated considering the premorbid function of the participant. Dementia was identified according to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition [16,17]. Only participants with impaired activities of daily living due to cognitive decline such as memory, orientation, and judgment were judged as having dementia, and participants with cognitive deterioration due to physical disability and depression were excluded [16-18]. The evaluation result was confirmed by a consensus panel conference that included a geriatric psychiatrist, clinical psychologist, and nurse. The presence of Alzheimer's dementia was determined according to the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association [17,19,20]. The diagnosis of Alzheimer's dementia includes both possible Alzheimer's dementia and probable Alzheimer's dementia. The diagnosis of MCI was made according to the Consensus Criteria of the International Working Group on MCI [17, 21]. If the participants did not meet the criteria for MCI

and dementia, they were defined as having normal cognition. In this study, diagnosis at baseline and diagnosis at the third follow-up were used.

Demographic and clinical variables

We recorded the age (years), sex, and education (years) of all the participants. Chronic illness burden was assessed using the Cumulative Illness Rating Scale (CIRS). The CIRS severity score was used to evaluate the severity of comorbidity: 0, denoted none; 1, mild; 2, moderate; 3, severe; and 4, denoted extreme comorbidity, and if there was more than one comorbidity, the average value was used [22].

Neuropsychological tests

As a neuropsychological test, the Korean version of the Mini Mental State Examination for Dementia screening (MMSE-DS) designed by Kim *et al.* [23] was used. The MMSE-DS reflects the demographic and cultural characteristics of the aged Korean population. The MMSE-DS consists of a total of 19 items such as time orientation (5 questions), place orientation (5 questions), memory registration and recall (2 questions), attention and concentration (1 question), language function (3 questions), constructional ability (1 question), and judgment (2 questions). The total score ranges from 0 to 30, the higher the score, the better the cognitive function.

Further, we assessed the participants' cognitive function using the CERAD-K [17]. The CERAD-K consists of the following sub-categories: J1, Verbal fluency test; J2, Boston Naming Test; J3, MMSE for Dementia Screening; J4, Word list memory test; J5, Constructional praxis test; J6, Word list recall test; J7, Word list recognition test; J8, Constructional recall test; and J9 A/B, Trail-Making Test A and B. The CERAD total score (TS) was calculated by adding the scores on J1－J7, according to a method described in a previous study [15,24,25].

As the data on the neuropsychological test scores at the third follow-up were not complete, we used the neuropsychological scores recorded at the baseline and at the second follow-up.

Measurement of glutathione and glutathione reductase levels

During baseline evaluation, 6,094 participants consented to blood collection and storage, and three pairs of plasma and serum cryovial tubes were stored in liquid nitrogen at −80°C in the Human Resources Bank of Kangwon National University Hospital. This study is a multi-center trial in which samples were collected separately at each center and stored at the Human Resources Bank of Kangwon National University Hospital. For GSH and GR analyses, information from the existing KLOSCAD registry data was used to select 20 samples from people with normal cognition and 20 samples from those with Alzheimer's dementia. After receiving approval from the Medical Life Research Institute of Kangwon National University Hospital, we obtained 40 plasma samples. Sample analysis was conducted by Iwon Corporation. For sample acquisition, after blood sampling through an EDTA tube, 0.3 cc (300 μml) of plasma was extracted by centrifuging at 1,000× g at 4°C for 15 minutes within 30 minutes. Later, the specimens were freeze-stored in the Human Resources Bank of Kangwon National University Hospital and had not been thawed prior to this study. The GSH level was analyzed in duplicate, using Avia System Biology's Glutathione ELISA Kit (OKEH02622) [26], to obtain the mean value. The measurement range of this kit was 1.25－80 ng/ml, and dilution was not performed, as specified in the manual. The GR level was also analyzed in duplicate, using Cloud Clone's SEB314Hu ELISA Kit [27], to obtain the mean value. The measurement range of this kit was 9.02－102.56 ng/ml. According to the manual, the sample was diluted 100-fold in PBS before the analysis.

Statistical Analyses

First, descriptive statistics for age, sex, education years, baseline CIRS severity score, baseline MMSE & CERAD total scores, baseline GSH and GR values of the normal cognition and Alzheimer's dementia groups were calculated. Next, multiple logistic regression analyses were conducted to determine whether the baseline GSH & GR levels had a significant association with the baseline diagnosis. Odds ratios (ORs) with 95% confidence interval (95% CI) and statistical p values were calculated. Next, multiple linear regression analyses were performed to determine whether the baseline GSH and GR levels were significantly associated with the baseline MMSE and CERAD total scores. In the linear regression analyses, standardized regression coefficient (β) and statistical p value were calculated. Before performing multiple linear regression analysis, the following assumptions were met. 1) Linearity:

When a scatter plot was drawn using the main independent variables GSH and GR and the main dependent variables MMSE and TS, linearity was satisfied. 2) Normality: The skewness of the dependent variables MMSE and TS was −1.181 and −0.239, respectively, and the kurtosis was 1.642 and −0.442, respectively, satisfying normality. 3) Independence: In collinearity statistics, the VIF (variance inflation factor) value was less than 1.5 for all variables, indicating no multicollinearity. 4) Homoscedasticity: When a residual scatter plot was drawn, the residuals were evenly distributed for all variables. Further, through longitudinal analysis, we attempted to determine whether baseline GSH and GR levels affected cognitive function longitudinally. Hence, logistic regression analysis was performed to determine whether the baseline GSH and GR were associated with the conversion rate from normal cognition to cognitive impairment (MCI or Alzheimer's dementia) at the third follow-up. Finally, a linear regression analysis was performed to determine whether the baseline GSH and GR levels were associated with changes in the MMSE and CERAD-TS scores from the baseline to the second follow-up. All of the above analyses were adjusted for sex and baseline age, education years, and CIRS severity score. Additionally, because a linear association between variables was not possible, the same analysis was repeated using a categorical variable in which the GSH and GR values were divided into lower and upper 50th percentiles and tertiles.

RESULTS

As per the descriptive statistics, the normal cognition group had a higher education level than the groups with Alzheimer's dementia and MCI. There was no significant difference in the GSH levels, but the GR level was significantly higher in the normal cognition group than in the Alzheimer's dementia group (Table 1).

The higher the GR level at baseline, the higher the probability of having normal cognition than of developing Alzheimer's dementia at the third follow-up (OR = 0.996, 95% CI: 0.992−0.999, p = 0.015), but there was no significant association with the GSH level (OR = 1.002, 95% CI: 0.816−1.246, p = 0.988). Even when using the categorical variables obtained by dividing the GR level into two (half) and three (tertile), the high GR group had a significantly higher probability of having normal cognition than developing Alzheimer's dementia (OR = 0.021, 95% CI: 0.002−0.258, p = 0.003; OR = 0.014, 95% CI: 0.001−0.313, p = 0.014, respectively). However, the GSH level had no significant effect (OR = 1.313, 95% CI: 0.292−5.905, p = 0.722; OR = 0.626, 95% CI: 0.086−4.558, p = 0.644, respectively) (Table 2).

The higher the GR level at the baseline, the higher the baseline MMSE score (β = 0.314, p = 0.031), but there was no significant association with the GSH level (β = 0.010, p = 0.942). In the analysis conducted with a categorical variable obtained by dividing the GR levels into half, the upper 50th percentile GR group had a significantly higher MMSE score than the lower 50th percentile group (β = 0.355, p = 0.008), but in the analysis conducted with the tertile categorical variables, no significant association was observed (β = 0.059, p = 0.627).

The average MMSE score was lower in the GR middle tertile group compared with the low tertile group, and the

Table 1. Characteristics of participants

Diagnosis	NC (n = 20)	AD (n = 20)	p value
Age (yr)	73.05 ± 4.97	73.15 ± 7.10	0.959
Sex, male	6 (30)	4 (20)	0.620
Education (yr)	8.70 ± 6.17	4.50 ± 4.43	0.018*
CIRS severity	1.42 ± 0.32	1.69 ± 0.49	0.043*
MMSE	26.05 ± 3.46	18.45 ± 6.60	< 0.001**
CERAD-TS	67.35 ± 11.10	38.60 ± 14.41	< 0.001**
GSH	14.00 ± 3.11	13.45 ± 3.86	0.623
GR	1,648.24 ± 359.97	1,319.12 ± 268.53	0.002**

Values are presented as mean ± standard deviation or number (%).
NC, normal cognition; AD, Alzheimer's dementia; CIRS, The Cumulative Illness Rating Scale; MMSE, The Mini Mental State Examination; CERAD-TS, The Korean version of the Consortium to Establish a Registry for Alzheimer's Disease total score; GSH, glutathione; GR, glutathione reductase.
*p < 0.05, **p < 0.01.

Table 2. Association between GSH, GR, and baseline diagnosis (NC or AD)

Stratification	Independent variables	Wald	Odds ratio (95% CI)	*p* value
Continuous variables	GSH	0.000	1.002 (0.806−1.246)	0.988
	GR	5.863	0.996 (0.992−0.999)	0.015*
Categorized	GSH lower half	Reference		
	GSH upper half	0.126	1.313 (0.292−5.905)	0.722
Categorized	GR lower half	Reference		
	GR upper half	9.101	0.021 (0.002−0.258)	0.003**
Categorized	GSH lower third	Reference		
	GSH middle third	0.949	0.366 (0.049−2.763)	0.330
	GSH upper third	0.214	0.626 (0.086−4.558)	0.644
Categorized	GR lower third	Reference		
	GR middle third	0.273	0.567 (0.067−4.771)	0.601
	GR upper third	7.296	0.014 (0.001−0.313)	0.014*

Adjust: sex, age, education, CIRS severity.
NC, normal cognition; AD, Alzheimer's dementia; CIRS, The Cumulative Illness Rating Scale; GSH, glutathione; GR, glutathione reductase; 95% CI, 95% confidence interval.
*$p < 0.05$, **$p < 0.01$

Table 3. Association between GSH, GR and baseline MMSE score

Stratification	Independent variables	Regression coefficient (95% CI)	Standardized regression coefficient	*p* value
Continuous variables	GSH	0.109 (−0.506 to 0.544)	0.010	0.942
	GR	0.006 (0.001 to 0.011)	0.314	0.031*
Categorized	GSH lower half	Reference		
	GSH upper half	0.760 (−2.860 to 4.380)	0.059	0.672
Categorized	GR lower half	Reference		
	GR upper half	4.538 (1.289 to 7.786)	0.355	0.008**
Categorized	GSH lower third	Reference		
	GSH middle third	4.340 (−0.206 to 8.886)	0.324	0.061
	GSH upper third	1.759 (−2.671 to 6.189)	0.129	0.425
Categorized	GR lower third	Reference		
	GR middle third	0.679 (−3.641 to 5.000)	0.059	0.672
	GR upper third	3.434 (−1.118 to 7.985)	0.252	0.134

Adjust: sex, age, education, CIRS severity.
MMSE, The Mini Mental State Examination; CIRS, The Cumulative Illness Rating Scale; GSH, glutathione; GR, glutathione reductase; 95% CI, 95% confidence interval.
*$p < 0.05$, **$p < 0.01$.

proportion of cognitive impairment was relatively high (Supplementary Fig. 1; available online). Meanwhile, no significant results were found in the analysis conducted with the categorical variables of GSH (half: β = 0.059, p = 0.672; tertile: β = 0.129, p = 0.425) (Table 3).

There was a trend that the higher the GR level at the baseline, the higher the baseline CERAD-K total score (β = 0.218, p = 0.073). However, this trend was not found with the GSH levels (β = −0.044, p = 0.702). In the analysis performed with GR as a categorical variable, the upper 50th percentile GR group had a significantly higher CERAD-K total score than the lower 50th percentile GR group (β = 0.270, p = 0.015), but it was not significant in the analysis performed with the tertile variable (β = 0.142, p = 0.308). There was no significant association in the analysis conducted with the categorical variables of GSH (half: β = −0.059, p = 0.614; tertile: β = 0.021, p = 0.877) (Supplementary Table 1; available online).

In the baseline normal cognitive group, the probability of conversion to MCI or Alzheimer's dementia after the third follow-up showed a decreasing trend as the GR level increased (OR = 0.993, 95% CI: 0.985−1.000, p = 0.063). Of the 20 participants with normal cognition at baseline, 6 developed MCI, and 1 developed Alzheimer's dementia at the third follow-up.

There was no significant association with the GSH level

(OR = 0.658, 95% CI: 0.379−1.147, p = 0.140). On categorizing the GR, the conversion rate of the upper 50th percentile was significantly lower than that of the lower 50th percentile (OR = 0.058, 95% CI: 0.088−1.147, p = 0.005). When it was divided into tertile, the conversion rate had a decreasing trend as the GR increased, but it was not statistically significant (OR = 0.147, 95% CI: 0.018−1.178, p = 0.071). No significant association was observed in the analysis performed with GSH as a categorical variable (half: OR = 1.182, 95% CI: 0.258−5.414, p = 0.829; tertile: OR = 0.395, 95% CI: 0.048−3.248, p = 0.387) (Supplementary Table 2; available online).

In the longitudinal analysis, baseline GR level was not significantly related to the changes in the MMSE scores from baseline to the second f/u period (β = −0.066, p = 0.707). Similarly, there was no significant association in the analysis with categorized GR levels (half: β = −0.068, p = 0.678; tertile: β = −0.110, p = 0.584). Baseline GSH was also not significantly related to the changes in MMSE scores (β = 0.148, p = 0.362), and categorized GSH showed no significance (half: β = 0.011, p = 0.947; tertile: β = 0.178, p = 0.369) (Supplementary Table 3; available online).

Baseline GR was not significantly related to CERAD-TS change between baseline and the second f/u period (β = 0.161, p = 0.306). Similarly, there was no significant association between categorized GR analyses (half: β = 0.109, p = 0.463; tertile: β = 0.061, p = 0.730). Baseline GSH was also not significantly related to CERAD-TS change (β = 0.040, p = 0.791), and this was also same to categorized variables (half: β = 0.073, p = 0.632; tertile: β = 0.098, p = 0.577) (Supplementary Table 4; available online).

DISCUSSION

This study showed that the higher the GR level in an individual, the significantly lower the probability of developing Alzheimer's dementia, and the objective cognitive function, as measured by the MMSE and CERAD-TS scores, increased as the GR increased. This association was not significant for GSH. In the longitudinal analysis, the higher the GR, the lower the conversion rate from normal cognition to cognitive decline, but there was no significant effect on changes in the MMSE and CERAD-K total scores. This association was not significant for GSH.

As GSH is converted to oxidized GSH (GSSG), it reduces oxidative stress by eliminating H_2O_2, which causes oxidative damage to brain cells. GSSG is converted back to the reduced form (GSH) by GR (Fig. 1). Therefore, GSH and GR are the main antioxidants that play a role in maintaining the redox balance in the human body. Since a neurodegenerative pathology is associated with oxidative stress [28], studies have attempted to determine whether these antioxidants have clinical potential as biomarkers of Alzheimer's disease. In previous studies, the GSH levels in the plasma and erythrocytes were significantly lower in the Alzheimer's dementia group [29] and MCI group [30] than in the normal cognition group. The GR level in the plasma and erythrocytes were also significantly lower in the Alzheimer's dementia [5,31] and MCI groups [5,28] than in the normal cognition group. In addition, in a longitudinal observational study, a high level of GSH significantly reduced the conversion rate from mild cognitive impairment to Alzheimer's dementia [10], while in another study, GSH did not show a protective effect against cognitive decline [13]. There was no study on the longitudinal effect of GR on cognitive function.

The results of this study are inconsistent with those of previous studies, wherein the higher the GSH level, the higher the probability of maintaining normal cognitive function. The reasons behind this contradiction are as follows: first, regarding the characteristics of the participant group, the average MMSE-DS score of the Alzheimer's dementia group was 18.45 in this study. Considering the average age (73.15 years) and average educational level (4.5 years), we can presume that the severity of dementia was very mild-to-mild [32].

Evidence suggests that GSH levels may vary based on the severity of Alzheimer's disease [9]; however, in this study, the Alzheimer's disease might not have been severe enough to show profoundly increased GSH levels. Therefore, it is possible that the difference in the GSH levels was not significant since the Alzheimer's dementia group had a relatively low level of cognitive deterioration. Further, in previous studies, there was a significant difference in the GSH levels between males with Alzheimer's dementia and normal cognition, but this was not observed for females [33]. In other words, in females, even if cognitive decline progresses, the decrease in the GSH level was not noticeable. The fact that females accounted for 80% of the Alzheimer's dementia group in this study might have

influenced the outcome. Lastly, since GSH is a substrate, not an enzyme, its ratio with oxidized GSSG may be more useful than its concentration in evaluating antioxidant capacity. Since the GSSG level was not measured in this study, the possibility could not be verified.

Consistent with the results of previous studies [5], GR had a protective effect against cognitive decline in this study. In particular, it is a novel finding that the conversion rate of the high GR group to MCI or Alzheimer's dementia was significantly low after 6 years of follow-up. However, the changes in the MMSE and CERAD-K scores from the baseline to the second follow-up period were not significantly different between the high GR and low GR groups. The reason for this could be the practice effect [31] occurring due to the measurements being obtained by the neuropsychological tests repeatedly every 2 years. In fact, the MMSE and CERAD-K scores had generally increased or were maintained during the second follow-up period. Therefore, there is a possibility that the neuropsychological examination did not sufficiently reflect the real cognitive changes because of the practice effect. In other words, if the sensitivity were increased using a diverse and complex neuropsychological test battery, a significant difference may have been observed in the follow-up.

Our results also showed that the average MMSE score was lower in the GR middle tertile group compared with the low tertile group, and the proportion of cognitive impairment was relatively high. It might be suggested that a heterogeneous group was mixed in the middle group; the 95% CI is wide. However, the regression analysis showed no significant differences between the low and middle groups (Table 3), which suggests that GR may have a protective effect above a certain threshold. Further investigation in large-scale studies remains warranted.

The limitation of this study is that the number of subjects in the target group was small, with only 20 patients with Alzheimer's dementia. Therefore, a high statistical power could not be achieved. The small sample size reduced the statistical power, especially in longitudinal studies. However, in our cross-sectional and longitudinal analysis with the conversion rate as dependent variables, the GR and GSH levels showed a significant difference in effect size (β) in addition to the p value. Hence, our result that the GR level is more useful than the GSH level as a significant predictor of cognitive decline can be considered meaningful.

Second, because participants with MCI at the baseline were not included in this study, the changes in the GSH and GR levels due to gradual cognitive decline were not reflected. In previous studies, it was found that the higher the GSH level, the lower the conversion rate to dementia in the non-demented group (normal cognitive + MCCI groups) [10]. Since the unique characteristics of the MCI group may have created a difference in these results, it is necessary to include groups with various cognitive functions in future studies.

Third, because the group was classified according to clinical diagnosis, it was not possible to confirm how Alzheimer's pathology in the brain affected the antioxidant system. In previous studies that examined post-mortem brain samples, CSF, and amyloid PET scans, the GSH [34-36] and GR [35-37] levels were associated with amyloid accumulation. Although the *in vivo* pathology could not be confirmed, the dementia in the patient group in this study corresponded to probable/possible Alzheimer's dementia according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria, and other types of dementia such as vascular dementia were excluded. The concordance between clinical and pathological Alzheimer's dementia is approximately 77%, and the concordance rate is lower in the older age groups [38]. The average age of the patients in our Alzheimer's dementia group was 73 years; therefore, we can assume that the concordance between clinical and pathological diagnoses was quite high in our study.

The results of this study suggest that the level of GR, a well-known antioxidant, can be used as a biomarker for detecting and predicting the progression of Alzheimer's dementia. On the other hand, the level of GSH, which is also a well-known antioxidant, did not show any significant association with the condition. The small sample size in this study reduced the statistical power, especially in longitudinal studies. However, this result should be confirmed in a long-term follow-up study including a large sample with diverse levels of cognition.

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■ Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

■ Author Contributions

Gihwan Byeon, Jin Hyeong Jhoo, and Sang-a Park were involved in the conceptualization of the study, analysis of the data, and drafting of the manuscript. Hyung-Chun Kim, Myoung-Nam Lim, and Jae-Won Jang assisted in the conceptualization the study, interpretation of the results, and editing of the manuscript. Jong Bin Bae, Ji Won Han, Tae Hui Kim, Kyung Phil Kwak, Bong Jo Kim, Shin Gyeom Kim, Jeong Lan Kim, Seok Woo Moon, Joon Hyuk Park, Seung-Ho Ryu, Jong Chul Youn, Dong Woo Lee, Seok Bum Lee, Jung Jae Lee, Dong Young Lee, Ki Woong Kim and So Jung Han assisted in the acquisition, analysis and interpretation of data. All authors critically revised the manuscript.

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