

Brain-derived neurotrophic factor and glutathione peroxidase as state biomarkers in alcohol use disorder patients undergoing detoxification

Shu-Yu Wu, MD, Chien-Yu Chen, MD, Tiao-Lai Huang, MD, Meng-Chang Tsai, MD*

Abstract

The pathophysiology of alcohol use disorder (AUD) is not totally clear. The aim of this study was to investigate the serum levels of brain-derived neurotrophic factor (BDNF) and oxidative stress markers in AUD patients during alcohol detoxification. Evaluation of changes in BDNF, glutathione peroxidase (GPX), catalase, superoxide dismutase, thiobarbituric acid reactive substances, 8-hydroxy 2'-deoxyguanosine, PCC and S100B were carried out.

14 AUD inpatients and 20 healthy control subjects were recruited for this study. The serum BDNF, S100B and oxidative stress markers were measured with assay kits.

Serum levels of catalase, GPX, PCC and 8-hydroxy 2'-deoxyguanosine were significantly higher in the AUD group subjects than in the controls ($P < .05$). However, BDNF levels were lower in the AUD group than in the controls ($P < .05$). After alcohol detoxification treatment, the GPX levels in the AUD group dropped ($P < .05$) and the BDNF levels rose ($P < .05$).

The results suggest that serum BDNF and GPX levels might be state biomarkers for AUD patients undergoing alcohol detoxification.

Abbreviations: 8-OHdG = 8-hydroxy 2'-deoxyguanosine, AUD = alcohol use disorder, BDNF = brain-derived neurotrophic factor, CAT = catalase, GPX = glutathione peroxidase, PCC = protein carbonyl content, SOD = superoxide dismutase, TBARS = thiobarbituric acid reactive substances.

Keywords: alcohol use disorder, brain-derived neurotrophic factor, catalase, glutathione peroxidase, oxidative stress, superoxide dismutase, thiobarbituric acid reactive substances

1. Introduction

Alcohol use disorder (AUD) not only affects patient physiologically and psychologically, but can also result in many social issues such as domestic violence, drunk driving or crime. The etiology of AUD has been attributed to genetic abnormality, autoimmune

regulation or even heredity.^[1,2] Oxidative stress,^[3] brain-derived neurotrophic factor (BDNF)^[4] and brain apoptosis^[5] also play roles in the cause of AUD.

Oxidative stress is defined as an imbalance between the production of free radicals and the antioxidant system.^[6] Alcohol-induced oxidative stress was shown to be related to impairment of antioxidant, including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities.^[7] 2-thiobarbituric acid reactive substance (TBARS) is widely used to quantify MDA,^[8] the level of which was found to decline to a normal range in AUD patients after treatment.^[7]

Reactive oxygen species (ROS) causes single- and double-stranded breaks in DNA, DNA bases, as well as sugar modification and covalent crosslinks in DNA protein.^[9,10] 8-hydroxydeoxyguanosine (8-OHdG) is the most prominent oxidative product resulting from damage to nuclear and mitochondrial DNA.^[11] PCC is also a marker of oxidative modification of proteins.^[12] However, S100B is a protein with the capacity to protect 5-HT neurons from damage caused by alcohol.^[13] BDNF regulates neuronal development, survival and plasticity.^[14,15] BDNF was vital in regulating drug addiction-related behavior^[16–18] through its effects on neuro-adaptation of neurons in brain reward area.^[19]

The brain is vulnerable to exposure to ROS since it is a major consumer of oxygen (20% of the body consumption) but has scanty antioxidant protection.^[20] It was found in a previous study that oxidative stress was not only related to the severity of schizophrenia in the acute phase^[21] but was significantly different in both bipolar I mania^[22] and major depression^[23] when

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Department of Psychiatry, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

* Correspondence: Meng-Chang Tsai, Department of Psychiatry, Chang Gung Memorial Hospital–Kaohsiung Medical Center, 123, Ta-Pei Rd, Niao-Sung, Kaohsiung 833, Taiwan, R.O.C. (e-mail: water444@ms21.hinet.net).

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compared to a healthy control group. Furthermore, indicators related to cell apoptosis such as S100B and Bcl-2 were related to the acute phase of schizophrenia^[24] and bipolar I mania.^[25,26] No studies have been done into the relationship of serum levels of biomarkers related to oxidative stress and cell apoptosis, or the relationship between brain tissue damage and biomarkers related to oxidative stress, in AUD patients.

The aim of this study was an investigation of the serum levels and activities of oxidative stress biomarkers, including SOD, GPX, CAT, TBARS, 8-OHdG, and PCC in AUD patients in the acute phase and to make a comparison with their state after treatment, as well as with healthy controls. The serum levels of BDNF and markers of apoptosis S100B were also measured and compared in the same groups.

2. Method

2.1. Patients and study design

The subjects were 14 AUD (12 male, 2 female) inpatients recruited over 1 year (November 2017 to October 2018) at the Kaohsiung Chang Gung Memorial Hospital. The inclusion criteria were as follows:

- (1) age from 20 to 65 years;
- (2) a diagnosis of AUD based on the DSM-V (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition);
- (3) no history of psychotic, bipolar, or substance dependence disorder except for alcohol or nicotine dependence disorder;
- (4) no physical illnesses, or physical illnesses under stable control.

The following data was collected for each patient: body mass index (kg/m²), age, serum SOD, CAT and GPX (parameters for anti-oxidative ability), S100B (marker for cell apoptosis), TBARS (marker for lipid peroxidation), 8-hydroxy 2'-deoxyguanosine (8-OHdG) (marker for DNA damage), PCC (marker for protein damage) and BDNF (marker related to brain cell damage) levels. The diagnosis of AUD was performed by a single board-certified psychiatrist for each subject.

The control group of 20 healthy subjects (18 male, 2 female) were recruited from Kaohsiung Chang Gung Memorial Hospital.

All the control subjects were free from any mental or physical disease and none were taking any prescription medication. The study was carried out at the Kaohsiung Chang Gung Memorial Hospital and was approved by the Hospital Institutional Review Board. All the participants signed an informed consent document after receiving an explanation of the study. The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice guidelines.

2.2. Laboratory data

A 15 mL blood sample was collected by venipuncture from each participant after an 8-hour fasting. The serum was immediately separated by centrifugation at 3000g for 10 minutes and the samples were stored at −80°C for further analysis. Commercially available enzyme-linked immunosorbent assay test kits were used to measure SOD, GPX, CAT, TBARS, PCC and 8-OHdG (Cayman Chemical Company, Ann Arbor), BDNF (Promega Corporation, WI) and S100B (Merck Millipore, Darmstadt, Germany). All the analyses were performed in the same laboratory by a trained technician.

2.3. Statistical analysis

The results were expressed as the means ± standard deviation. Comparisons between the oxidative stress markers, S100B and BDNF in both the AUD patients and control group were assessed using the *t*-test. Changes in biomarker levels in the AUD patients after alcohol detoxification treatment were analyzed by the paired *t*-test. A *P* value of less than .05 was used as an indication of statistical significance.

3. Results

The average duration of alcohol misuse among the 14 AUD participants recruited for the study was 31 ± 6.4 years. Table 1 shows the demographic and biochemical data of the AUD and control group subjects. All 14 AUD inpatients were followed up, and their serum SOD, GPX, CAT, TBARS, PCC, 8-OHdG, S100B and BDNF levels or activities were measured at the end of the hospitalization period. The *t*-tested data showed that the

Table 1
Demographic data, Oxidative-stress markers and BDNF of alcohol use disorder patients and healthy controls.

Variable	Patients (n=14)	Controls (n=20)	P-value
Age, yr	48.4 ± 6.0	44.8 ± 3.4	
BMI, kg/m ²	23.2 ± 3.1	24.9 ± 3.5	
Duration of hospitalization, d	13.4 ± 9		
Duration of alcohol use, yr	31.14 ± 6.36		
CiWA-Ar scores	8.21 ± 4.53		
SOD, U/ml	4.8 ± 2.2	4.3 ± 1.1	<i>F</i> = 0.053, <i>P</i> = .431
CAT, nmol/min/mL	132.1 ± 91	73.3 ± 26.4	<i>F</i> = 0.004, <i>P</i> = .033*
GPX, nmol/min/mL	190.2 ± 23.7	163.3 ± 17.3	<i>F</i> = 0.478, <i>P</i> = .001*
TBARS, μmole/L	9.5 ± 5.4	6.8 ± 2.3	<i>F</i> = 0.166, <i>P</i> = .049
PCC, nmol/mg	3.6 ± 1.8	1.2 ± 0.4	<i>F</i> < 0.001, <i>P</i> < .001*
8-OHdG, pg/mL	4361.7 ± 941.4	3615.6 ± 1099.4	<i>F</i> = 0.391, <i>P</i> = .047*
S100B, pg/mL	26.4 ± 15.8	22.7 ± 12.2	<i>F</i> = 0.511, <i>P</i> = .45
BDNF, ng/mL	11.2 ± 4.4	17.7 ± 2.4	<i>F</i> = 0.006, <i>P</i> < .001*

Plus-minus values are given as mean ± standard deviation.

8-OHdG = 8-hydroxy 2'-deoxyguanosine, BDNF = brain-derived neurotrophic factor, BMI = body mass index, CAT = catalase, GPX = glutathione peroxidase, PCC = protein carbonyl content, SOD = superoxide dismutase, TBARS = thiobarbituric acid reactive substances.

* *P* < .05.

Table 2**Mean changes and pair-*t* test in serum markers after treatment.**

Variable (n=14)	CIWA-Ar scores	SOD (U/mL)	CAT (nmol/min/mL)	GPX (nmol/min/mL)	TBARS (μmole/L)	PCC (nmol/mg)	8-OHdG (pg/mL)	S100B (pg/mL)	BDNF (ng/mL)
Baseline	8.21 ± 4.5	4.8 ± 2.2	132.1 ± 91	190.2 ± 23.7	9.5 ± 5.4	3.6 ± 1.8	4361.7 ± 941.4	26.4 ± 15.8	11.2 ± 4.4
Endpoint	0	4.7 ± 1.7	94.7 ± 57.4	166.0 ± 24.4	6.8 ± 1.6	3.8 ± 2.0	4056.2 ± 974.8	19.9 ± 9.2	14.1 ± 3.4
<i>P</i> value	-	.848	.238	.006*	.074	.630	.106	.099	.004*

Plus-minus values are given as mean ± standard deviation.

8-OHdG = 8-hydroxy 2'-deoxyguanosine, CAT = catalase, GPX = glutathione peroxidase, HAM-D = Hamilton Depression Rating Scale, PCC = protein carbonyl content, SOD = superoxide dismutase, TBARS = thiobarbituric acid reactive substances.

* *P* < .05.

CAT, GPX, PCC and 8-OHdG serum levels in AUD patients were significantly higher than those in the control group (*P* < .05), while BDNF serum level was lower in the AUD group (*P* < .05). Table 2 shows the serum biomarker levels or activity at baseline and end points in the AUD patients. Analysis using the paired *t*-test, showed a significant decrease in GPX activity and an increase in BDNF level in the AUD subjects after alcohol detoxification treatment. The other markers (SOD, CAT, TBARS, PCC, 8-OHdG and S100B) showed no significant changes.

4. Discussion

One of the important findings in this study was that AUD patients in acute alcohol detoxification treatment had a significantly higher GPX, CAT, PCC and 8-OHdG, but lower BDNF serum levels than the healthy control subjects.

The serum level of GPX, another antioxidant defense for the removal of hydrogen peroxide, was found to be higher in the AUD patients before alcohol abstinence, than in the control group. The result was similar to that of previous studies,^[27,28] and suggested the AUD patients in our study had adapted to oxidative stress related to alcohol use. However, in some other studies, GPX was found to be lower in subjects used to alcohol than in control groups.^[29,30] The differences may have been the result of severe liver disease related to alcohol use, since chronic alcohol exposure would impair antioxidant defense in both hepatic tissue and the blood.^[31,32] Moreover, chronic alcohol exposure is related to alteration of the selenium (Se) balance as well as GPX, a seleno-protein, this suggests that assay of the Se/MDA ratio might be useful in the evaluation of underlying liver disease.^[33]

It was also found that the CAT level was higher in the AUD group than in control group in this study. Others^[7] reported similar results although they seemed to be of no significance. However, an animal study showed clear dose-dependent elevation of CAT under chronic alcohol consumption.^[34] Research indicated that alcohol induced over-expression of cytochrome P450 enzymes 2E1 may also augment CAT activity^[35] which would support our findings.

Our study showed higher serum 8-OHdG levels in the AUD subjects than in the controls. This finding was seen to agree with the results of an earlier study where alcohol-withdrawal severity was positively correlated with 8-OHdG level.^[36] Alcohol exposure was reported to cause oxidative DNA damage in a dose-dependent relationship clearly demonstrated by the 8-OHdG level.^[37] The byproducts of the metabolism of alcohol, ROS and acetaldehyde, are implicated in the induction of DNA damage such as oxidative modifications,

acetaldehyde-derived DNA adducts and cross-links.^[38] In vitro experiments with human peripheral lymphocytes showed that acute ethanol-induced DNA damage is reversible through auto-repair,^[39] which may explain the declining trend of 8-OHdG level after alcohol detoxification seen in this study.

Another finding was that the PCC level was higher in the AUD group than in control group. This is supported by previous research,^[40,41] and such correlation was specific and more frequently found in chronic alcohol abusers with liver disease than in those without liver disease.^[33] One of the reasons for impaired liver function in AUD patients is excessive oxidative stress from ethanol or acetaldehyde resulting in damage or the protein oxidation of hepatic cells.^[42] Animal studies have shown increased PCC level among alcohol-fed rats^[43] while other studies have revealed the correlation of PCC level to the amount daily alcohol intake.^[44] The increased PCC level correlates with oxidative changes to proteins, which may cause impairment of multiple domains as the inhibition of enzymatic activity, increased susceptibility of proteins to proteolysis and altered immunogenicity.^[45]

The essential finding of our study was that AUD patients have significantly different serum GPX (*P* = .006) and BDNF (*P* = .004) levels after hospital treatment compared to the baseline. The alteration of GPX activity from a high baseline to a significantly lower 1 after alcohol withdrawal was similar to findings in previous studies^[27,46] where the alcohol withdrawal period was about 2 to 3 weeks.^[27,29,30] The GPX level in AUD patients after the cessation of alcohol use seemed normalized in our study. However, existing evidence about the GPX level at the end point being normalized was inconclusive^[29] or even lower than in the control group.^[7,27]

Our data indicated that serum BDNF level was lower in the AUD group at the base line than in the controls, which agrees with prior studies.^[3,47,48] Serum BDNF levels among alcohol dependent patients were found to be higher than the baseline after a 6-month period.^[48] During alcohol abstinence, the increased serum levels of BDNF indicated neuronal remodeling, which played a role in AUD relapse.^[48] Although it was found that BDNF mRNA expression increased during the acute phase of alcohol use, it decreased after long-term exposure to alcohol.^[19] The variation of the BDNF gene, baseline serum r-GT and relapse count in AUD were related to the alteration of BDNF after treatment.^[49] AUD patients with liver and pancreas disease have even lower BDNF levels^[50] while others claimed no difference,^[4] the relationship between alcohol use and BDNF remains inconclusive. In previous study, BDNF levels were increased in heroin-dependent patients who received methadone maintenance treatment.^[51] However, increased BDNF were also found in

patients with cannabis dependence.^[52] These results indicated BDNF might be related to neuronal plasticity and reward system, but the mechanism still needs further research.

The results of this study showed there was a tendency towards a higher TBARS level in the AUD group compared to the control, but this was not statistically significant. Other studies reported that patients suffering from alcohol dependence have higher MDA or TBARS levels (escalating lipid oxidation) than normal control groups.^[7,30,36,53,54] However, previous studies may support our findings, in suggesting that AUD patients in this study had less severe liver disease. More lipid oxidation was found in patients with liver disease than without amongst alcohol-tolerant patients^[33] while the level of lipid oxidation was also related to the severity of hepatic injury.^[31] In addition, the TBARS level declined after cessation of alcohol use in the AUD group and was lower than that in the control group.^[30,54,55]

Most studies showed significantly increased plasma SOD during withdrawal than was present in control groups.^[30,46,55] On the other hand it was shown that SOD activity in the erythrocytes of alcohol dependent patients was lower than in the control group, either in the acute or abstinent phases.^[54] Some studies found that SOD activity went down after 2 to 3 weeks without alcohol compared with the baseline.^[27,29] However, the effect of chronic alcohol use as well as the severity of hepatic illness remain unclear, but are still important considering their association with attenuated SOD activity.^[7] However it is 1 of the factors where our data showed no significant difference between the 2 groups.

The limitations to our study were:

- (1) The sample size of the AUD group was small and there may have been some statistical bias;
- (2) The gender ratio was not balanced and the sample size was not large enough for an analysis of variance to be done;
- (3) Covariance such as the severity of hepatic disease, the serum Se level, dietary habit and details of alcohol use (amount, type, frequency and duration) were not taken into account.

5. Conclusion

Our results suggest that serum BDNF and GPX levels might be AUD state biomarkers and useful in alcohol detoxification treatment. In the future, additional human studies with larger group are needed to search the similarities and differences between genders or ethnicity. Trying to explore the detailed mechanism to confirm our findings.

Author contributions

Huang TL and Tsai MC conceptualized and designed the study and formulated the hypotheses and analytical strategies. Wu SY, Chen CY and Tsai MC wrote the manuscript. All authors contributed to the preparation and approved the final manuscript.

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