Original Article

Oxidative Stress During Alcohol Withdrawal and its Relationship with Withdrawal Severity

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ABSTRACT

Background: Oxidative parameters are altered during alcohol withdrawal and are said to contribute towards withdrawal symptoms in alcoholic patients. **Aims:** To study levels of five selected oxidative parameters during alcohol withdrawal state and after treatment of the withdrawal state and to assess the association of the oxidative parameters with the severity of alcohol withdrawal. **Materials and Methods:** This was a case-control study done in a De-addiction clinic of a tertiary teaching centre, Southern India. 50 persons having alcohol withdrawal symptoms were included. The oxidative stress parameters malondialdehyde, protein carbonyl, glutathione peroxidase, superoxide dismutase and catalase were assessed in during the withdrawal phase and again after the withdrawal had subsided. The same oxidative stress parameters were measured in the control group. **Statistical analysis:** Statistical analysis was done using SPSS version 17.0. One way ANOVA and Pearson correlation test were used for finding the association between the oxidative stress parameters levels and the severity of alcohol withdrawal. Multiple linear regression analysis done to predict variables associated with level of oxidative parameters. **Results:** During alcohol withdrawal the pro-oxidant malondialdehyde was elevated compared to that in the control group. Among the antioxidant enzymes the superoxide dismutase was higher and catalase was lower than the control group levels. After remission of the alcohol withdrawal both malondialdehyde remained higher and superoxide dismutase lower than in the control group. The levels of oxidative stress parameters not correlated with the severity of alcohol withdrawal. **Conclusions:** oxidative stress parameters show changes during alcohol withdrawal and during the remission of withdrawal. However, levels of oxidative stress parameters not correlated with the severity of withdrawal.

Key words: *Alcoholism, alcohol withdrawal, catalase, glutathione peroxidase, malondialdehyde, oxidative stress, protein carbonyl, superoxide dismutase*

INTRODUCTION

Alcoholism is a synonym for alcohol dependence syndrome, a severe form of alcohol use disorder.[1] When some persons with alcohol dependence reduce or stop taking alcohol, they develop a set of symptoms and signs called as alcohol withdrawal syndrome. Various mechanisms have been put forward to explain the development of withdrawal symptoms. The most accepted explanation for the withdrawal phase is increased excitatory glutamatergic and reduced inhibitory GABAergic neurotransmission in the central nervous system (CNS).[2] Oxidative stress is hypothesized to be one mechanism mediating these withdrawal symptoms.[3]

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Several studies have addressed the role of oxidative stress with alcohol use and during withdrawal. The results of these studies are conflicting in that some oxidative stress parameters as either increased, decreased, or unaltered in alcohol withdrawal.[4-7] Most studies examined the role of one or other oxidative stress parameter in isolation. Thus, the full picture of oxidative stress could not be understood with the available literature.

Current study aimed at measuring five oxidative stress parameters during alcohol withdrawal and finding their association with severity of withdrawal. Specifically, we chose to estimate following oxidative stress markers: Malondialdehyde (MDA), protein carbonyl, glutathione peroxidase, superoxide dismutase (SOD), and catalase.

MATERIALS AND METHODS

This study was conducted in a tertiary teaching hospital in southern India, between October 2011 and April 2013. Institute Ethics Committee approved the study.

It was a cross-sectional case control study with 50 subjects in each group. Sample of convenience taken for the current study. To be included as a case, subjects should have:

(i) Fulfilled alcohol dependence syndrome with or without nicotine dependence as per International Classification of Disease, 10th revision, Diagnostic Criteria for Research (ICD-10 DCR; Chapter V: Mental and behavioral disorders) criteria,
(ii) Between 18-60 years of age,
(iii) Male gender and
(iv) Accompanied by key informant.

To be included as control, subject should be:

(i) Between 18-60-year-old male,
(ii) Not fulfill criteria for alcohol dependence in the past 1 year and
(iii) Not consumed alcohol at any time in the past 1 month.

They were matched for age (± 5 years) and nicotine dependence as per ICD-10 DCR criteria to the study group. Those with pre-existing unstable medical conditions including hepatic encephalopathy, pre-existing comorbid psychiatric illnesses and pre-existing diagnosed medical conditions that could affect oxidative stress, autoimmune diseases, malignancy, diabetes mellitus and hypertension excluded from both the groups. A semi-structured proforma was used to collect information on socio-demographic factors such as age, gender, occupation, religion, socioeconomic status, number of years of dependence, amount of alcohol consumed per day on average, presence of nicotine dependence, presence of medical and psychiatric complications, family history of alcohol dependence. Clinical Institute Withdrawal Assessment-Alcohol revised (CIWA-Ar) was used for assessing severity of alcohol withdrawal.[8] The CIWA-Ar scale measures 10 symptoms. Scores of less than 8 indicate minimal to mild withdrawal. Scores of 8 to 15 indicate moderate withdrawal (marked autonomic arousal); and scores of 15 or more indicate severe withdrawal (impending delirium tremens). The assessment requires 2 minutes to perform. A cut-off score of eight was used to differentiate between presence and absence of alcohol withdrawal syndrome/phase.

A written informed consent obtained for participation in the study. Subjects coming with alcohol withdrawal admitted to de-addiction unit. At the time of initial evaluation withdrawal severity was rated on scale CIWA-Ar and screened for inclusion and exclusion criteria. Blood samples were taken before starting the pharmacotherapy for baseline values of oxidative stress parameters during the withdrawal phase. A score of more than 8 on CIWA-Ar scale at the initial time of assessment before starting the pharmacotherapy defined the withdrawal phase. Pharmacotherapy mainly consisted of benzodiazepines (Diazepam or lorazepam), multivitamin injections and omeprazole and was carried out as done routinely and no intervention modified for the study purpose. The severity of alcohol withdrawal was serially monitored by CIWA-Ar scale every 8 hours. Another blood sample was taken for assessment of oxidative parameters once the CIWA-Ar score was less than 8 and remained stable for at least 8 hours apart that defined the remission of the withdrawal phase.

Five millilitre of venous blood collected from each subject of both the groups. In the cases, it was collected for the second time during the remission of the withdrawal phase as defined earlier. The samples centrifuged at 2500 rpm for 5 minutes. The plasma was separated and stored in aliquots at −20°C. The plasma MDA estimated by the method described earlier.[9] The concentration of MDA calculated using the molar extinction co-efficient ($1.56 \times 10\,5$ M/L/cm) and molecular weight of MDA and was expressed in micromole/litre. The plasma protein carbonyl contents measured according to the modification of Levines method.[10] Concentration of protein carbonyl calculated using molar extinction coefficient (21×10^3 M/L/cm) and molecular weight of protein carbonyl and expressed in nanomoles/mg of protein.

The glutathione peroxidase activity in erythrocytes estimated by method described by previous researcher.[11] The values expressed in U/g Hb. The catalase activity in erythrocytes estimated by the method of Aebi, 1984.[12] The catalase activity expressed as rate constant (k/ml). For the determination of SOD activity, u/Zn SOD levels were measured by sandwich enzyme-linked

immunosorbent assay (ELISA) using the commercial kit and standard protocol (19160 SOD determination kit; Sigma-Aldrich, Switzerland). SOD expressed in U/ml.

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) statistical software for windows version 17.0. Descriptive statistics (mean ± standard deviation) was used to summarize the study findings. For comparing the means of the three groups namely control group, during withdrawal and after withdrawal had subsided one-way analysis of variance (ANOVA) was used. Values of oxidative stress parameters beyond two standard deviations from the mean were considered as outliers. The association between the baseline oxidative stress parameters levels and the severity of alcohol withdrawal as measured on CIWA-Ar scale measured using Pearson's correlation test. Multiple linear regression analysis was done to examine the relationship between various potential predictors and the levels of individual oxidative stress parameters at baseline for cases. To consider the result to be statistically significant *P* value has to be less than 0.05 for two-tailed tests.

RESULTS

Current study had a sample size of 100. The size of each group (cases and controls) was 50. Sample groups matched for age and nicotine dependence. The mean age of cases group was 37.94 ± 7.79 years (mean ± SD) and the mean age of controls was 36.62 ± 8.66. The average duration of alcohol dependence was 9.20 ± 5.06 years. On an average, the cases consumed 18.02 ± 7.14 units of alcohol per day. The baseline severity of alcohol withdrawal as measured on CIWA-Ar was 14.94 ± 4.18. A total of 28 had nicotine dependence among cases and controls. The interval time between the first sample and second sample was 36.12 ± 25.85 hours. Pearson's correlation test was conducted to examine the relationship between the five oxidative stress parameters levels at baseline and the baseline alcohol withdrawal score on CIWA-Ar scale. None of the oxidative stress parameters significantly correlated with baseline withdrawal severity [Table 1]. A one-way ANOVA was used to test for differences of individual oxidative parameters among the three groups namely controls, during the withdrawal state and after the remission of alcohol withdrawal state. MDA levels differed significantly across the three groups, F (2,147) = 15.961, $P < 0.001$. *Post hoc* analyses using the Scheffé *post hoc* analysis showed that the MDA significantly elevated during withdrawal and after remission of alcohol withdrawal state when compared to the control group [Figure 1]. MDA levels decreased during the remission of alcohol withdrawal state compared to its level during the withdrawal phase but still remained significantly high compared to the control group. PCO levels differed significantly across the three groups, F (2,147) = 4.674, $P = 0.011$. *Post hoc* analyses using the Scheffé test showed that PCO levels during the withdrawal state did not differ significantly from the control group. And protein carbonyl was significantly elevated after remission of alcohol withdrawal state when compared to control group [Figure 2]. Glutathione peroxidase levels did not differ significantly across the three groups, F (2,147) = 0.320, $P = 0.727$. A one-way ANOVA test showed that SOD

Table 1: Correlation of oxidative stress parameters to severity of alcohol withdrawal measured at baseline by CIWA-Ar score

Oxidative stress parameter	Pearson's r	Significance level
Malondialdehyde	0.000	0.996
Protein carbonyl	−0.142	0.325
Glutathione peroxidase	0.173	0.230
Superoxide dismutase	−0.110	0.446
Catalase	−0.124	0.392

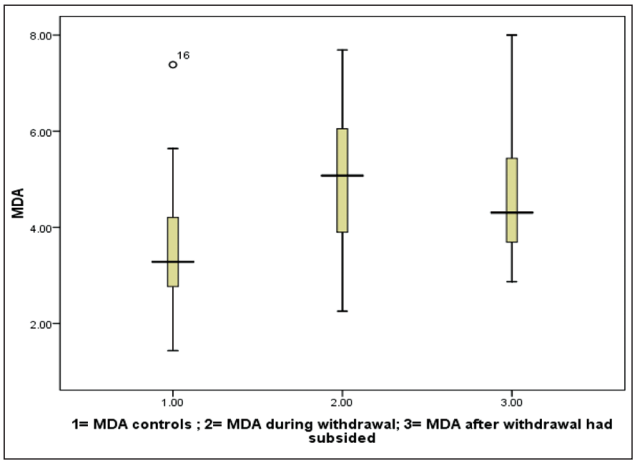

Figure 1: Comparison of Malondialdehyde levels among control group, during withdrawal state and after remission of withdrawal state

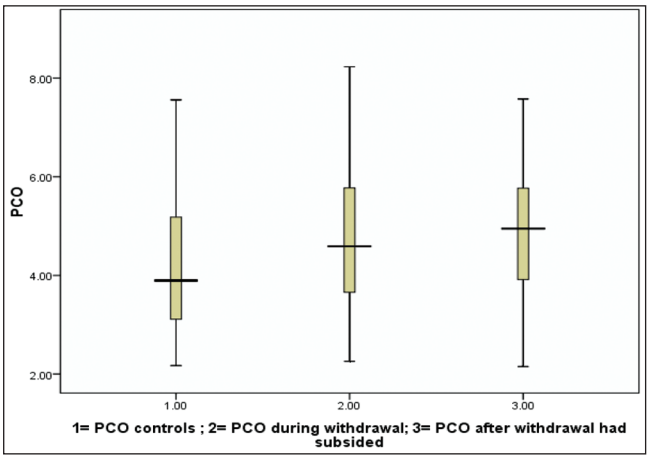

Figure 2: Comparison of Protein Carbonyl (PCO) levels among control group, during withdrawal state and after remission of withdrawal state

levels differed significantly across the three groups, F (2,147) = 57.949, $P < 0.001$. *Post hoc* analyses using the Scheffé test showed that superoxide dismutase during the withdrawal state was significantly higher when compared to levels in control group and levels after remission of alcohol withdrawal state. SOD level after remission of alcohol withdrawal state was significantly lower when compared to the control group [Figure 3]. Similarly, catalase levels differed significantly across the three groups, F (2,147) = 6.357, $P = 0.002$. *Post hoc* analyses using the Scheffé *post hoc* analysis showed that catalase was significantly lower during the withdrawal state when compared to levels in the control group. Catalase level after remission of alcohol withdrawal state was not significantly different when compared to the control group or from levels during the withdrawal state [Figure 4].

Then multiple linear regression analysis was conducted to examine the relationship between various oxidative stress parameters and potential predictors such as age, average use of alcohol per day and duration of alcohol dependence. Level of MDA significantly correlated with duration of dependence (Pearson's r = −0.314) and was not correlated with the other two parameters age and average use of alcohol per day [Table 2]. The multiple regression model with all three predictors was weakone [$R^2 = 0.120$, F(3, 46) = 2.100, $P = 0.113$]. The duration of dependence had significant negative regression weights, indicating people with higher duration of dependence were expected to have lesser malondialdehyde levels. None of the three variables was able to predict other parameters like protein carbonyl, glutathione peroxidase, SOD, catalase.

DISCUSSION

In this study, we measured oxidative stress parameters in 50 persons who presented in alcohol withdrawal state. Then they underwent treatment as usual with benzodiazepines, multivitamin injections and omeprazole. We serially monitored the severity of alcohol withdrawal by a standard scale (CIWA-Ar). Same oxidative stress parameters reassessed during the remission of withdrawal state. We recruited 50 controls who were matched with cases for age and nicotine dependence.

MDA was significantly elevated in alcohol withdrawal and remained higher even after the remission of withdrawal state. Protein carbonyl was significantly elevated after emission of withdrawal state. Glutathione peroxidase was not different during alcohol withdrawal or after remission of alcohol withdrawal. Superoxide dismutase was significantly higher during the withdrawal phase and fell lower than the level in the control-group after remission of withdrawal state. Catalase was significantly lower during withdrawal and was similar to control group after remission of withdrawal state. The levels of oxidative stress parameters studied viz. malondialdehyde, proteincarbonyl, glutathione peroxidase, superoxide dismutase and catalase did not correlate well with the severity of alcohol withdrawal as measured on CIWA-Ar.

Table 2: Summary of multiple regression analysis of variables predicting Malondialdehyde level during withdrawal

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
Constant	4.996	1.109		4.506	0.000
Age	0.014	0.028	0.073	0.488	0.628
Average use of alcohol per day(units/day)	0.027	0.028	0.135	0.970	0.337
Number of years of alcohol dependence	−0.103	0.043	−0.360	−2.386	0.021

Dependent Variable: Malondialdehyde during withdrawal

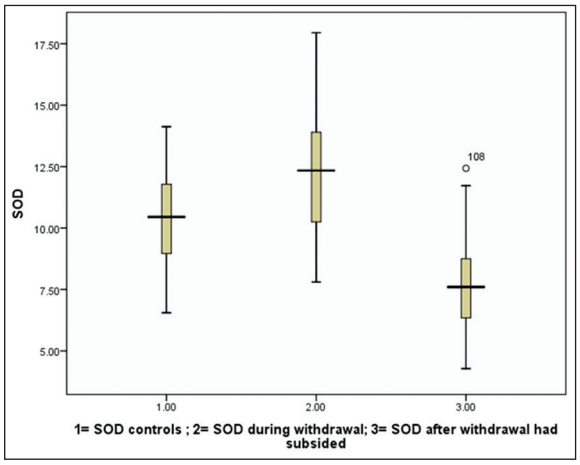

Figure 3: Comparison of superoxide dismutase levels among control group, during withdrawal and after remission of withdrawal state

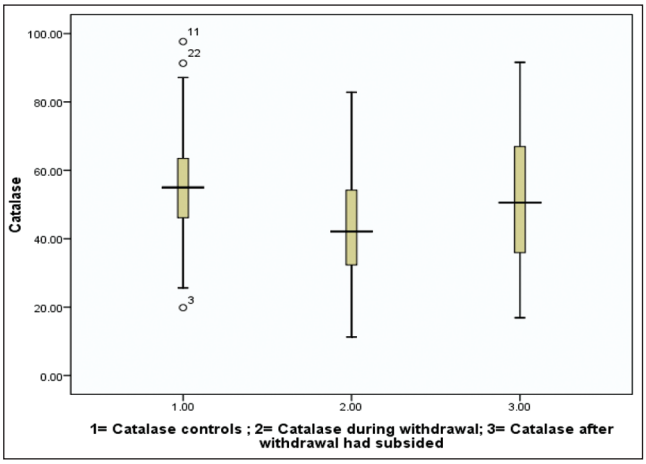

Figure 4: Comparison of catalase levels among control group, during withdrawal and after remission of withdrawal state

In the present study, we found that plasma MDA was significantly elevated in alcohol withdrawal state. This finding has been consistently reported by previous studies.[3-7] We did not find association between MDA level and severity of alcohol withdrawal which is in contrast with previous reports.[6,7] Many studies have also compared MDA during active use of alcohol and after abstinence is achieved. The usual trend seems to be that MDA is high during active alcohol use and gradually decreases over time during an abstinence period.[13,14] We saw the same trend but during this small period of observation it did not decrease significantly during the remission of the withdrawal symptoms.

In the present study plasma protein carbonyl during the withdrawal phase was not different compared to the control-group levels. Previous study reported lower levels of protein carbonyl during the withdrawal phase in the CSF from alcohol dependent subjects.[3] It is possible that the changes in CSF may not be reflected in the peripheral blood in the immediate withdrawal period. So far no study has assessed the plasma level of protein carbonyl in alcohol withdrawal. The difference between the findings of the two studies may be due to inherent differences between the basal levels of PCO in the brain (1.5 to 6.4 ng/mg protein) versus human plasma (0.4 to 1.0 ng/mg protein). Moreover, the method of estimation of PC varies from one study to another and comparability of the two studies may be compromised (Dalle-Donne *et al.*, 2003).[15]

In the present study, erythrocyte glutathione peroxidase was not different during alcohol withdrawal when compared to control group levels. This finding in our study is similar to that reported in the literature.[4] While another study had reported lower glutathione peroxidase in moderate drinkers who abstained from alcohol.[16] However, this study was not clear on presence or absence of withdrawal symptoms. So it is difficult to conclude the comparability of that study with our study. Glutathione peroxidase was found to be decreased during alcohol withdrawal in some previous studies.[6,17] This discrepancy in the findings of these studies and the present study can be explained by different sampling methods and sources of glutathione peroxidase.

In the present study, plasma SOD during withdrawal was significantly increased during alcohol withdrawal state similar to the previous report.[4] It was different from the findings reported by others who found that superoxide dismutase was lowered during alcohol withdrawal state.[6,17] The differences can be attributed to varying methodology, different samples for testing (plasma versus serum), different age-groups of the sample population and different levels of smoking in the study population. In the present study, catalase was significantly lower during withdrawal compared to the control-group in line with the similar reporting by earlier studies.[6] On the other hand, Wozniak *et al*., 2008 did not find any change in catalase activity during alcohol withdrawal.[17] In the present study, we found that the MDA levels decreased after remission of withdrawal from baseline but still remained significantly higher when compared to the control group. This was in line with the previous research, meaning that the oxidative stress continues to be present even though the clinical features of alcohol withdrawal had subsided.[5]

Thus, the present study confirms the presence of oxidative stress in alcohol withdrawal. Treatment methods that target deranged oxidative stress parameters are more likely to be fruitful. The relative importance of each of the oxidative stress parameters in alcohol withdrawal requires clarification.

The lack of correlation between the oxidative stress parameters and alcohol withdrawal severity was unexpected. Oxidative stress is not a mediating or an etiological factor in symptoms of alcohol withdrawal or levels of oxidative stress markers in the periphery may not reflect the oxidative stress in the brain, in which case CSF will be the ideal source of sample. Main strength of this study was that it included a number of (five) oxidative stress parameters including two pro-oxidants and three antioxidant enzymes and monitored the change in the same parameters.

Study results are limited by the fact that we did not consider the effect of diet and other possible confounding factors like nicotine that possibly affect oxidative stress markers. Concurrent use of drugs like multivitamins and benzodiazepines might have influenced the oxidative stress parameters during remission of alcohol withdrawal state and we did not control for specific vitamins, their dose and route of administration. Future study need to be designed to measure and account for variables that can influence oxidative stress markers in alcohol withdrawal.

Future study designs should address the pathophysiological role of oxidative stress in alcohol withdrawal. Adequate control of confounding factors should be done in future studies to show a causal role of oxidative stress to alcohol withdrawal. Also studying oxidative stress in the brain rather than in the peripheral areas will lend more credence to the role of oxidative stress in the pathophysiology of alcohol withdrawal. Lastly, role of antioxidants in the treatment of alcohol withdrawal needs exploration.

To conclude, in the current study we found that during alcohol withdrawal phase MDA and superoxide dismutase were elevated, catalase was lowered and glutathione peroxidase and protein carbonyl levels were not different when compared to controls. Superoxide dismutase subsided significantly compared to its high levels found during withdrawal. The oxidative stress parameters studied were not correlated with the severity of withdrawal.

The findings of the study are consistent with the hypothesis that oxidative stress would be increased during alcohol withdrawal but not with the other hypothesis that oxidative stress markers would be correlated with the severity of alcohol withdrawal.

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