Homocysteine as a biomarker for predicting alcohol withdrawal syndromes: Insight from a retrospective cohort study

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Abstract

Background

The alcohol withdrawal syndrome is a group of symptoms and signs that usually arise within 24-48 hours of abrupt alcohol cessation or significant reduction in consumption in alcohol dependent individuals. The most severe complication of alcohol withdrawal syndrome is delirium tremens, which may be preceded or complicated by seizures. In recent years, there is a growing interest in the role of serum homocysteine levels in chronic alcohol dependent patients undergoing withdrawal. A number of studies have shown that alcohol dependent patients have elevated serum homocysteine levels and that it can be considered to be a risk factor for severe withdrawal states. The purpose of this study was to evaluate the serum homocysteine levels in alcohol withdrawal syndromes.

Methodology

The study was a retrospective cohort of alcohol dependent patients (N=49) admitted with a diagnosis of delirium tremens (DT) (n=28) and simple alcohol withdrawal (SW) (n=21) as per the ICD-10 classification. A semi-structured pro-forma was used to gather information from the file records covering both socio-demographic, clinical and biochemical variables. The historical variables included proposed risk factors like amount of daily consumption of alcohol, duration of abstinence and past history of complicated withdrawal. The serum homocysteine, vitamin B12 and folate levels of all patients sent within 24 hours of admission were also collected. Comparisons between clinical and biochemical variables were made between the two groups using Chi-square test and Student's T test.

Results

The mean age of patients of DT was 33.07 ± 8.24 years while that of patients with SW was 37.19 ±7.60 years. The mean duration of alcohol use in dependent pattern was 10.00 ±3.00 years in DT compared to 8.66 ±4.76 years in SW. The laboratory investigations revealed a mean serum homocysteine level of 21.09 ± 12.81in patients presenting with DT as compared with 15.06 ± 6.17 in patients having SW and this difference was statistically significant ($p<0.05$). The serum folate levels differed significantly in the two groups with patients in DT having a lower mean value of 6.30 ± 3.67 as compared to patients with SW 10.23 ± 7.04. Patients with DT with withdrawal seizures (n=18) had statistically non-significant rise in their mean homocysteine levels as compared to patients with DT without withdrawal seizures (n=10).

Conclusion

The results of this study highlight the potential role of homocysteine as a new biomarker to predict the severity of alcohol withdrawal syndromes. However, there is a need for further research to prove whether targeting homocysteine levels will be of benefit with respect to alcohol related disorders.

Key words: Homocysteine; Delirium Tremens; Simple alcohol withdrawal; Alcohol withdrawal seizure; Folate, Vitamin B12.

1. Introduction

The alcohol withdrawal syndrome is a group of symptoms and signs that usually arise within 24-48 hours of abrupt alcohol cessation or significant reduction in consumption in alcohol dependent individuals. Mild alcohol withdrawal state includes autonomic hyperactivity, insomnia, tremors, nausea or vomiting and anxiety as prominent symptoms. The signs and symptoms of alcohol withdrawal have been postulated to be due to the enhanced NMDA receptor functioning, reduced GABAergic transmission and dysregulation of the dopaminergic system (Lingford-Hughes & Nutt, 2003). The altered numbers and functions of NMDA (particularly NR1 and NR2B subtypes) and $GABA_a$ receptors resulting from chronic alcohol exposure may be partly responsible for the severe alcohol withdrawal states (Qiang, Denny, & Ticku, 2007). The most severe complication of alcohol withdrawal syndrome is alcohol withdrawal delirium tremens (DT), which may be preceded or complicated by seizures (Mainerova et al., 2015). While most patients end up having mild withdrawal, about 24% to 32% of patients might land in DT which has a mortality of 0 to 8 % (Lee et al., 2005). As DT is a clinical state with significant morbidity and mortality, a number of studies have been undertaken to study the predictor of DT (Berggren et al., 2009; Eyer et al., 2011; Goodson, Clark, & Douglas, 2014). Hypokalemia, thrombocytopenia and past episodes of severe alcohol withdrawal have been implicated as predictors of severe alcohol withdrawal (Goodson et al., 2014). However, in recent years there is a growing interest in the role of serum homocysteine levels in chronic alcohol dependent patients undergoing withdrawal. A number of studies have shown that alcohol dependent patients have elevated serum homocysteine levels and that it can be considered to be a risk factor for severe withdrawal states (Bayerlein et al., 2005; S. Bleich, Degner, Wiltfang, et al., 2000; Kim, Kim, Bae, Park, & Kim, 2015). Clinical experience suggests that the best way to manage DT is with prompt identification and treatment. Thus, identification of potential

risk factors that can predict the development of DT becomes an empirical tool in prevention of this clinical state. The purpose of this study was therefore to evaluate the serum homocysteine levels in alcohol withdrawal syndromes with a null hypothesis that there will be no difference in the serum homocysteine levels in simple alcohol withdrawal (SW) and in delirium tremens.

2. Methodology

The present study was carried out in the de-addiction unit of a tertiary care neuropsychiatric hospital. It was a retrospective cohort study of alcohol dependent patients admitted with a diagnosis of DT and SW in the de-addiction ward from June 2015 to December 2015. To be included in the study, the individuals had to either fulfill a discharge diagnosis of Mental and behavioural disorders due to use of alcohol; withdrawal state with delirium (F10.4) or Mental and behavioural disorders due to use of alcohol; withdrawal state; uncomplicated (F10.30) as per the ICD-10 diagnostic guidelines (World Health Organization, 1992). Discharge diagnosis rather than admission diagnosis was used to increase the sensitivity of cohort identification.

2.1. Treatment Period

All patients were attended to by a qualified psychiatrist at the time of admission and were evaluated in detail within 24 hours by a trained psychiatrist specializing in de-addiction. All blood investigations were sent within 24 hours of admission. Patients were treated with a fixed or as needed dosage of benzodiazepines depending on their clinical state. Additional medications like vitamin supplementation were only given when considered clinically necessary and after the samples were drawn for laboratory investigations. For all patients admitted, a hospital record was created or the current admission notes were added to their previous records. Detailed history regarding alcohol use and other relevant history along with general physical examination and mental state examination were documented in the hospital records. As the current cohort consisted of a group of patients presenting in DT, history

regarding substance use was obtained additionally from an informant. Since most of the patients were residing with their family, reliable history could be obtained from family members.

2.2. Evaluation of records

The patient records (N=49) included in the study were divided into 2 groups: SW (n=21) and DT (n=28). Those patients with co-morbid medical conditions like cardio-vascular disease, hypertension, liver disorders and endocrinal disorders were excluded from the study, as they would have confounded the results. Additionally, patients already on vitamin supplementation before admission or those taking other medications causing hyperhomocysteinemia like methotrexate, metformin, insulin (Desouza, Keebler, McNamara, & Fonseca, 2012) were also excluded from the study. An inpatient admission for less than 24 hours was considered as insufficient time period for a detailed evaluation of the patients and therefore such patients were also not included in the study. A semi-structured pro-forma was used to gather information from the file records covering both socio-demographic, clinical and biochemical variables. The historical variables included proposed risk factors like amount of daily consumption of alcohol, duration of abstinence, past history of complicated withdrawal, history of co-morbid substance use amongst others. The serum homocysteine, vitamin B12 and folate levels of all patients sent within 24 hours of admission were also collected. Since the tests for serum pyridoxine levels were not available at our center, therefore this variable could not be studied.

2.3. Statistical Analysis

The data was analyzed using the Statistical Package for Social Sciences, version 20 (SPSS, Inc. Chicago, IL) for Windows 10. Comparisons between clinical and biochemical variables were made between the two groups using Chi-square test and Student's T test and p-value of less than 0.05 was considered significant while evaluating the data. Given the retrospective nature of this study, it was exempted from the assessment by the institutional ethics committee.

3. Results

The descriptive analysis of the data showed that all the patients were males in both the SW and DT group. The mean age of patients of DT was 33.07 ± 8.24 years while that of patients with SW was 37.19 ±7.60 years. The mean duration of alcohol use in dependent pattern was 10.00 ±3.00 years in DT compared to 8.66 ±4.76 years in SW. As shown in Table 1, 64.28% of patients who presented in DT consumed more than 720 ml of alcohol per day which was significantly (p=0.03) greater than in patients with SW. 64.30 % patients of DT had a withdrawal seizure in the 72 hours of presentation in the hospital.

Table 1: The clinical variables and their comparison between cases of delirium tremens (DT) and simple alcohol withdrawal (SW) by X^2 Chi Square test

S. NO	VARIABLE		CASES (N=49) DELIRIUM TREMENS (DT n=28) (%)	CASES (N=49) SIMPLE WITHDRAWAL (SW n=21) (%)	X^2	p
1.	Amount of alcohol consumption/day	< 720 ml	35.71	66.66	4.60	**0.03***
		>720 ml	64.28	33.33		
2.	Duration from last drink	< 72 hours	42.86	100.00	17.81	**0.00***
		>72 hours	57.14	0.00		
3.	Withdrawal seizure in past 72 hours	Present	64.30	0.00	**21.34**	0.00*
		Absent	35.70	100.00		
4.	Past history of withdrawal seizures	Present	42.90	23.80	1.92	0.17
		Absent	57.10	76.20		
5.	Past history of delirium tremens	Present	28.60	19.00	0.59	0.44
		Absent	71.40	81.00		

*p VALUE SIGNIFICANT AT < 0.05

The laboratory investigations as depicted in Table 2 revealed a mean serum homocysteine level of 21.09 ± 12.81 in patients presenting with DT as compared with 15.06 ± 6.17 in patients having SW and this difference was statistically significant. The serum vitamin B12 level in the two groups was in normal range and the difference was not significant. However, the serum folate levels differed significantly in the two groups with patients in DT having a lower mean value

of 6.30 ± 3.67 as compared to patients with SW 10.23 ± 7.04. Both the DT and SW groups were comparable and the difference not significant statistically on the parameters of BMI, number of cigarettes smoked per day and other co-morbid substance used. Furthermore, patients with DT were separated into subgroups: those with withdrawal seizures and those without withdrawal seizures. There was no significant difference in the serum homocysteine and serum vitamin B12 levels in these two subgroups (Table 3).

Table 2: The clinical and biochemical variables and their comparison between cases of delirium tremens (DT) and simple alcohol withdrawal (SW) by Independent student's t test

S. NO	VARIABLE	CASES (N=49)		P
		DELIRIUM TREMENS (DT) n=28 Mean (SD)	SIMPLE WITHDRAWAL (SW) n=21 Mean (SD)	
1.	Age (in years)	33.07 (8.24)	37.19 (7.60)	0.08
2.	Duration of Alcohol use (in years)	10.00 (3.00)	8.66 (4.76)	0.23
3.	S. Homocysteine (µmol/L)	21.09 (12.81)	15.06 (6.17)	**0.05****
4.	S. Vitamin B12 (ng/L)	297.28 (169.88)	242.00 (112.04)	0.20
5.	S. Folate (µg/L)	6.30 (3.67)	10.23 (7.04)	**0.01****

** P VALUE SIGNIFICANT AT < 0.05

Table 3: The comparison of biochemical variables between cases of delirium tremens (DT) and simple alcohol withdrawal (SW) by ANOVA

S. NO	VARIABLE	DELIRIUM TREMENS (DT) with Withdrawal seizure (n=18) Mean (SD)	DELIRIUM TREMENS (DT) without Withdrawal seizure (n=10) Mean (SD)	SIMPLE WITHDRAWAL (SW) (n=21) Mean (SD)	f	p
1.	S. Homocysteine	21.72 (13.26)	19.96 (12.58)	15.06 (6.17)	3.94	0.53
2.	S. Vitamin B12	302.11 (178.24)	288.60 (162.60)	242.00 (112.04)	1.67	0.20
3.	S. Folate	6.13 (4.13)	6.60 (2.82)	10.23 (7.04)	6.44	**0.01*****

*** p VALUE SIGNIFICANT AT < 0.05

4. Discussion

The present study provides evidence that serum homocysteine levels are elevated in persons undergoing alcohol withdrawal syndromes. The normal range in adults is usually 5–15 μM, with a mean level of about 10 μM (Refsum, Ueland, Nygård, &Vollset, 1998). This study showed that alcohol dependent patients had elevated serum homocysteine levels both in SW and DT and the difference between the mean homocysteine values was also statistically significant in these two groups. Furthermore, both the subset of patients of DT with or without withdrawal seizures had elevated serum homocysteine levels as compared to SW. In the recent past, multiple studies have evaluated the role of serum homocysteine levels in alcohol dependent patients. Bleich et al (S. Bleich, Degner, Wiltfang, et al., 2000) reported that moderate hyperhomocysteinemia was seen in patients who underwent alcohol withdrawal. They also added that the levels of homocysteine decreased as the days of abstinence increased with homocysteine levels at day 1 of withdrawal having a strong correlation with blood alcohol concentration. Bayerlein et al (Bayerlein et al., 2005) found that hyperhomocysteinemia predicts first onset alcohol withdrawal seizures while Kim et al (Kim et al., 2015) suggested that elevated serum homocysteine levels can predict the development of delirium tremens in patients who have alcohol withdrawal seizures.

This study also showed that the serum folate levels were depleted significantly more in patients with DT. Laboratory studies involving patients and experimental primates have demonstrated the association of chronic alcoholism with the intestinal malabsorption, decreased hepatic uptake and increased urinary excretion of folic acid thus resulting in folate deficiency (Halsted, Villanueva, Devlin, & Chandler, 2002). Surprisingly, the serum vitamin B12 levels in our patients with delirium tremens were greater than the levels in simple withdrawal. This trend has been seen in other studies also (Cravo et al., 1996; Cylwik, Czygier, Daniluk, Chrostek, & Szmitkowski, 2010) but an accurate reason is yet to be found. Present study

also showed that patients of DT who had withdrawal seizures had marginally higher levels of serum homocysteine as compared to those patients of DT without withdrawal seizures though this difference was not significant.

The metabolism of homocysteine is interlinked with the availability of vitamin B6, B12 and folates in the body (Kang, Wong, & Norusis, 1987). Homocysteine is a sulfur-containing amino acid derived from the metabolism of methionine which is converted to S-adenosylmethionine (SAM). Homocysteine is metabolized by two biochemical pathways: remethylation and transsulfuration (Kim et al., 2015). The remethylation pathway is dependent on the vitamin B12 and folate levels and is responsible for the basal homocysteine levels. Folic acid is converted to 5-methyltetrahydrofolate which provides the methyl group for remethylation of homocysteine to methionine and vitamin B12 acts as a co-factor in this remethylation reaction. The transsulfuration pathway is on the other hand dependent on the pyridoxine levels. The hyperhomocysteinemia found in alcoholics is primarily due to the deranged remethylation that is partly caused by an alcohol-induced deficiency of vitamin B12 and folate (Stefan Bleich et al., 2005). Elevation of serum homocysteine may also be due to inherited disorders of metabolism or other genetic factors that influence the enzyme pathways involved in its metabolism. 5,10-methyltetrahydrofolate reductase (MTHFR) is an important enzyme in the homocysteine metabolism and catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the predominant circulating form of folate (Brustolin, Giugliani, & Félix, 2010). Functional polymorphism in the MTHFR results in reduced enzymatic activity leading to decreased concentrations of folate in serum, plasma, and red blood cells, and mildly increased plasma total homocysteine concentration (Jacques et al., 1996). This elevation of homocysteine levels has been implicated in contributing to the symptoms of alcohol withdrawal state though an exact mechanism for the cause of delirium tremens is still not known. Homocysteine and a product of its metabolism, homocysteic acid, acts as excitatory molecules resulting in

overstimulation of the NMDA receptors (Lipton et al., 1997). Homocysteic acid is a putative neurotransmitter and acts as an endogenous agonist at NMDA receptors. Additionally, homocysteine has also been found to act as an agonist at the glutamate binding site of NMDA receptor (Lipton et al., 1997) leading to an imbalance in the excitatory-inhibitory transmission. Homocysteine has been implicated to play a role in not just alcohol withdrawal states, but has been proposed as a biomarker for alcohol withdrawal seizures (S. Bleich, Degner, Bandelow, et al., 2000).

In summary, there is strong evidence that chronic alcohol use in dependent pattern is associated with derangement in the homocysteine metabolism. This ethanol induced hyperhomocysteinemia and the accompanying excitatory neurotransmitter misbalance may partly mediate the wide array of signs and symptoms seen in alcohol withdrawal states.

The index study had however a number of limitations. Firstly, since it was a retrospective cohort study with a relatively smaller number of patients, establishing the predictive value of homocysteine as a biomarker for development of delirium tremens was difficult. The absence of information on other clinical parameter like blood alcohol concentration, serum pyridoxine levels was also a limiting factor.

5. Conclusion

The role of homocysteine in severe alcohol withdrawal, particularly delirium tremens and withdrawal seizures, cannot be ignored. Since ethanol mediated hyperhomocysteinemia can be treated by vitamin B supplementation, there lies a potential to study the use of folate and vitamin B12 in treating severe alcohol withdrawal. Thus, serum homocysteine level at the time of admission might be a useful tool in screening for patients at risk for severe withdrawal. Further controlled and prospective study would be able to study this potential biomarker more accurately. Prospective intervention studies are required to show whether the folate administration reduced the incidence of complications of alcohol withdrawal or not and whether targeting homocysteine levels is of benefit with respect to alcohol withdrawal syndromes.

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