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Impact of Antabuse Treatment on Dyslipidemia and Oxidative Stress Levels in Chronic Alcoholism

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Background: Chronic alcoholics are known to have dyslipidemia, which can aggravate organ dysfunction along with increased oxidative stress due to alcohol metabolism. We are studying the impact of Antabuse treatment in reversing the damages caused by chronic alcoholism at metabolite level.

Results: This is hospital based cross sectional case control study, with 34 chronic alcoholic patients and 20 Antabuse treated patient for alcoholism. The mean plasma MDA (oxidative stress marker) level among the chronic alcoholics was 12.3 ± 4.3 µmol/L and in Antabuse treated group was 2.8 ± 0.3 µmol/L. Chronic alcoholic group had significantly elevated lipid profile then Antabuse treated group suggesting dyslipidemia in alcoholic group in our study population.

Discussion: We observed positive correlation between oxidative stress marker and Lipid profile. Dyslipidemia and oxidative stress levels were significantly lower in Antabuse treated patients suggesting early treatment of such addicts and complete abstinence from consumption showed reversal of the metabolic derangement caused by chronic alcoholism.

Key words: Alcoholism, Oxidative Stress, Dyslipidemia, Malondialdehyde, Disulfiram

Alcoholism is one of the major public and social health problems. Alcohol pre-se directly affect two organ system, brain and heart; however, liver is the major organ effected by chronic alcoholism due to its involvement in detoxification of alcohol via ADH/ALD system, cytochrome p450 and other microsomal oxidative enzymes. Alcohol in general, metabolized to acetate. Alcohol is oxidized to acetaldehyde by alcohol dehydrogenase enzyme (ADH) and then to acetate by aldehyde dehydrogenase enzyme (ALD). Acetaldehyde is known toxic molecule to tissue if accumulated in larger amount. The activity of aldehyde dehydrogenase is slower compared to alcohol dehydrogenase, hence acetaldehyde, accumulates in liver and leads to gradual cellular death (Vasudevan *et al.*, 2019). Alcohol produces 7 Kcals of energy on metabolism , but this energy is not useful, since the NADH produced during the metabolism of Alcohol inhibits glycolysis and kerb cycle due to increased NAD/NADP ratio inside the cell, which in turn leads to increased lipolysis, altered lipid metabolism and accumulation of triacylglycerol and free fatty acid. Triglyceride and free fatty acid are substrate for many peroxidation reactions resulting in free radical production and propagation (Das and Vasudevan, 2005). Hence, chronic alcoholism resulting in increased acetaldehyde and enhanced oxidative stress over time leading to cellular death and tissue damage of various organs including cardiovascular, central nervous system and liver damage (Das and Vasudevan, 2005). Central nervous system is more prone for alcoholic damage not only to the direct effect of alcohol but due to free radical damages to its huge lipid content leading to neuronal death (Das and Vasudevan, 2005). Chronic alcoholism has shown to decreased the total brain weight when compared with normal non-alcoholic subjects and the amount of brain atrophic degeneration correlates with the frequency and volume of alcohol taken over a long time (Harper and Matsumoto, 2005). This leads to behavioral changes and social problems related to chronic alcoholism and enhance oxidative stress due to chronic alcoholism.

We aimed to study the metabolic variations and oxidative stress level in chronic alcoholics group and group of alcoholics who underwent treatment with disulfiram as part of their deaddiction carried out for a minimum of at least six months period, to see the impact of Antabuse treatment in reversal of metabolic derangement seen in chronic alcoholism.

MATERIALS AND METHODS

This study is a hospital based cross sectional case control study carried out in Vinayaka Mission Medical College and hospital, Karaikal, Puducherry. Thirty-four chronic alcoholics under the age of 45 years were selected from alcohol deaddiction center OPD after their informed consent. Twenty age and gender matched apparently healthy individuals who underwent treatment for alcoholism with disulfiram for atleast complete six months were taken as controls. Participants with Diabetics, smoking history, hypertensive patients, Acute liver dysfunction patients and patients on treatment for any diseases were excluded. Study was conducted after Institutional ethical committee approval and informed consent from all the participants.

Body Mass Index (BMI) and Clinical parameters like Blood pressure, pulse rate, height, weight were recording from all the participants. 5 ml blood was collected for Routine biochemical investigations like fasting and postprandial plasma glucose level, fasting lipid profile such as Serum Total Cholesterol (TC), serum Triglyceride (TG), HDL-Cholesterol (HDLc), LDL-Cholesterol (LDLc) and liver function tests along with enzyme Gamma Glutamyl Transferase (GGT) and Malondialdehyde. GGT along with other routine biochemical investigation were analyzed by commercial kits in autoanalyzer (maglumi) using maglumi fully automated analyzer kit. Serum LDL-cholesterol and VLDL- cholesterol was calculated using Fried Wald's formula and cardiac risk factor ratios like TC/HDL, LDL/HDL were also mathematically calculated. Plasma Malondialdehyde (MDA) marker for oxidative stress was estimated using Esterbauer and Steinberg method (Esterbauer *et al.*, 1993), based on principle of lipid peroxides getting condensed with 1 methyl- 2-phenyl indole (MPI) under acidic conditions resulting in formation of coloring agent, which was measured using spectrophotometer at 586 nm. The normal cut off is of MDA is taken as 4 µmol/L. Statistical analysis was done

using SPSS IBM v 22. Student 't' test is used for comparing means and Pearson correlation was used for correlation between the biochemical markers.

RESULTS

This study was conducted on a group of chronic alcoholics who attended deaddiction center OPD for treatment and group of alcoholics who underwent treatment with disulfiram (250 mg once daily dose) under supervision for 21 days and then on maintenance dose with regular follow up and counselling for six weeks and not have consumed alcohol for atleast 6 moths. The mean age of cases and controls participants were 37.3±5 and 42.1±5 years respectively. Mean duration of alcohol intake in cases was 9.4 ± 4.3 years.

The mean values of lipid profile among cases and controls showed a significant variation and in chronic alcoholics group they were in dyslipidemic range. The mean serum HDL-c in cases was 37.7 ± 9.1 mg/dl and in controls 50.8 ± 2.1 mg/dl. LDL-c was 124.8 ±28.2 mg/dl in alcoholics group and 97.2 ±9.7 mg/dl in controls group (Table 1). Similarly, the cardiac risk factor ratios such as TC: HDL –c ratio and LDL-c: HDL-c ratio were also elevated in cases compared to the controls (Table 1). The difference in all the mean serum values of lipid parameters and risk ratios except for Total Cholesterol were highly significant between cases and controls (Table 2).

The marker of chronic alcoholism serum GGT was increased in cases, with mean serum levels of 90.0 ± 27.6 IU/L compared to mean serum levels in controls (24.4±5 IU/L) (Fig 1). Interestingly, BMI also observed to be above the cut off value and was in the range first degree obesity with mean value of 26.4 ± 3.8 kg/m^2 in cases.

The oxidative stress marker MDA was significantly increased in cases with mean value of 12.3 ± 4.3 µmol/L whereas in controls it was 2.8 ± 0.3 µmol/L, p=0.024 (Fig 1, Table 2). We observed significant positive correlation of oxidative stress parameter MDA with cardiac risk factor ratios such as TC: HDL –c ratio and LDL-c: HDL-c ratio (Table 3)

Table 1. Biochemical parameters in the study subjects

PARAMETERS	CASES (34)	CONTROLS (20)
FBS (mg/dl)	82.6 ± 15	85.5 ± 8.5
PPBS (mg/dl)	122.4 ± 24.1	125.1 ± 9.4
TC (mg/dl)	189.5 ±40.4*	164.1 ± 9.3
TGL (mg/dl)	134.9 ±33.11*	81.4 ± 9.8
HDLc (mg/dl)	37.7 ± 9.1	50.8 ±2.1*
LDLc(mg/dl)	124.8 ± 28.2*	97.2 ± 9.7
VLDLc(mg/dl)	27 ± 6.6*	16.3 ±1.9
TC: HDLc	5 ± 0.6*	3.2 ± 0.2
LDLc: HDLc	3.3 ± 0.5*	1.8 ± 0.2
AST IU/L	35.8 ±15.5	19.8 ±5.1
ALT IU /L	35.6 ± 9.1	19.6 ±3.7
ALP IU/L	100.4 ±18.9*	59.7 ±17.8
ALBUMIN (gm/dl)	3.7 ± 0.6	4.4 ±0.2
A:G Ratio	1.5 ± 0.5	1.8 ±0.2
GGT (IU/L)	90.9 ± 27.6*	24.4 ±5.0
BMI (kg/m^2)	26.43 ± 3.8	23.1 ± 2.1
MDA (µmol/L)	12.3 ± 4.3*	2.8 ± 0.3

Mean values of the study parameters in the cases (alcoholic group) and control (Antabuse group) is presented along with the standard division. *p<0.05 is taken as statistically significant difference among the group.

FBS: Fast blood sugar, PPBS: post prandial blood sugar, TC: Total Cholesterol, TGL: Triglyceride, HDLc: HDL-Cholesterol, LDLc: LDL-Cholesterol GGT: Gamma Glutamyl Transferase, ALP: Alkaline Phosphatase, ALT: serum alanine aminotransferase, AST: aspartate aminotransferase, BMI: Body Mass Index, MDA: malondialdehyde

Table 2. Mean Comparison of study parameters among Cases and Controls

Parameters	TC	TGL	HDLc	LDLc	TC/HDL ratio	LDL/HDL ratio	VLDL	GGT	MDA
Student t-Value	0.0081	4.574	5.55	8.1357	8.57	4.8733	3.6306	10.63	2.355
p – Value	0.9935	**0.00065***	**0.0004***	**<0.001***	**<0.001***	**0.0002***	**0.0009***	**<0.0001***	**0.024***

Student 't' was used to compare the mean values of study parameters among alcoholic and Antabuse treatment group. *p<0.05 is taken as statistically significant.
TC: Total Cholesterol, TGL: Triglyceride, HDLc: HDL-Cholesterol, LDLc: LDL-Cholesterol, GGT: Gamma Glutamyl Transferase, VLDL: Very Low-density Lipoprotein, MDA: malondialdehyde

Table 3. Correlation of MDA with cardiac risk factor markers

MDA	TC	HDLc	LDLc	TC/HDLc	LDLc/HDLc	GGT
r^2- Value	0.0941	0.0185	0.0875	0.386	0.438	0.1083
p – Value	0.5	0.91	0.622	**0.02***	**0.034***	0.54

Pearson correlation coefficient was used for correlation of MDM with other lipid prolife and cardiac profile markers and corresponding r2 value and p value is given above. *p<0.05 is taken as a statistically significant correlation.
TC: Total Cholesterol, HDLc: HDL-Cholesterol, LDLc: LDL-Cholesterol, GGT: Gamma Glutamyl Transferase, MDA: malondialdehyde

DISCUSSION

Alcohol is metabolized in human body by two distinctive mechanism, most common being by enzymes alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALD) combination and the second less common system involving enzymes cytochrome p450 2E1 (CYP2E1) and catalase which also breaks down alcohol to acetaldehyde. The Acetaldehyde is toxic molecule in the alcohol metabolism leading to various adverse effect seen in alcoholism from liver dysfunction, phycological and behavioral problems to cancer. The metabolism of alcohol via cytochrome system happens in people who are chronic and heavy drinkers. Metabolism of alcohol by Cytochrome p450/catalase system generates free radical apart from producing acetaldehyde, which leads to additional damage to the tissues. Thus, alcoholism causes acetaldehyde accumulation and elevation of oxidative stress leading to tissues damage. Antabuse treatment involves, facilitating alcohol abstinence, thus prevent many adverse effects of alcoholism. Disulfiram administrated as part of Antabuse treatment is a carbamate derivative and acts by inhibiting aldehyde dehydrogenase. It leads to alcohol deterrence by causing acetaldehyde syndrome. When alcohol is ingested after administration of disulfiram, blood acetaldehyde concentrations are increased, followed by flushing, systemic vasodilation and nausea which ultimately makes the person to keep away from alcohol (Huffman and Stern, 2003). Absence from alcohol consumption itself has a potential to reduce oxidative stress induced due to chronic alcoholism.

Oxidative stress is an imbalance between antioxidant and pro-oxidant levels, where the balance is altered towards pro oxidants and free radicals generation (Halliwell *et al.*, 1992). Though liver metabolizes the major portion of ingested alcohol by ADH and ALD enzymes into non-toxic product acetate, however, beyond certain threshold level metabolism of alcohol shifts towards Cytochrome p450 and catalase (Savolainen *et al.*, 1993). This shift leads to production of free radicals and free radical mediated damages to the tissues. Many previous studies have confirmed the fact that free radical induced lipid peroxidation products like malondialdehyde (MDA), damages directly or indirectly the lipocytes and hepatocytes resulting in fibrosis of liver (Deshpande *et al.*, 2013) and existing literature has very well established that alcohol induces

lipid peroxidation enhancing oxidative stress and cellular damage (Situnayake *et al.*, 1990). In our study, we observed higher mean MDA levels in alcoholic group and positive correlation of MDA with cardiac risk factor ratios such as TC: HDL –c ratio and LDL-c: HDL-c ratio, suggesting the damaging role of free radical generation induced by alcohol and potential cardiac risk. It is reported that lipid peroxidation leads to oxidatively modified LDL that ultimately results in the gradual formation of atherosclerotic lesions (Shanmugapriya *et al.*, 2013; Singh *et al.*, 2013; Situnayake *et al.*, 1990). Oxidized LDL when deposited in vascular walls, releases biological active compounds continuously which in turn irritates surrounding endothelial cell layer leading to inflammation and fat deposition (Rajendran *et al.*, 2013). It is also very well known that oxidized LDL contains active cytotoxic lipid peroxidation products like certain aldehydes (MDA, 4-HNE), which can diffuse into the cells and are responsible for the free radical damage of blood vessels and other cellular pathology (Rajendran *et al.*, 2013; Shanmugapriya *et al.*, 2013). In our study we have observed increased MDA levels in alcoholic group and significant decrease in the MDA levels in Antabuse treatment group, suggesting the role of absenteeism from alcohol helps in rebalancing the oxidative stress. We observed increase in cardiac risk factor ratio (TC: HDL –c ratio and LDL-c: HDL-c ratio) in alcoholic group, suggesting the alcoholism is causes dyslipidemia also. Since dyslipidemia and peroxidation of lipids have direct consequences to atherosclerosis, preventing the formation of free radicals will thus, limit the anthogenesis in peripheral vascular tissues and might reduce the complications and progression.

Alcohol plays a major role in the pathogenesis and progression of hepatic damage and Gamma Glutamyl Transferase (GGT), an enzyme derived from the plasma membrane of hepatocytes is widely accepted as a biomarker of Alcoholic Liver Disease can be a usefulness marker for monitoring the effects of ethanol on the liver (Singh *et al.*, 2013). In our study we have observed significantly higher GGT levels in alcoholic group compared to group staying away from alcoholic ingestion. Thus, providing an alternative marker to assess the effectiveness of Antabuse treatment

CONCLUSION

The adverse impact of alcohol consumption results in decreasing man power, disability-adjusted life year (DALY) and overall productivity of the nation and its development. Chronic alcoholism induces metabolic derangements such as increased oxidative stress and dyslipidemia. Antabuse treatment rebalancing of oxidative stress and causes reversal of dyslipidemia upon absenteeism from alcohol. Since the percentage of social drinking is increasing among adolescents and middle age population, the oxidative stress marker MDA, alcohol consumption marker GGT should be used as a regular investigative parameters among alcoholics for their risk assessment along with but also as a monitoring tool to assess the effectiveness of during Antabuse treatment.

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The Research involves human participants and appropriate clearance was taken from Vinayaka Mission Medical College and hospital, Karaikal, Puducherry (VMMC/ethical/125/2016) and informed consent was taken from all the participants of the study.

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