Original Paper

A Study of the Levels of Serum Malondialdehyde, Alpha Tocopherol, Beta Carotene and Vitamin C after Micronutrient Supplementation during Alcohol Rehabilitation

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ABSTRACT

This study was undertaken to test the effect of a 21-day supplementation of antioxidant nutrients on biochemical indicators of lipid peroxidation, and vitamin levels in alcohol-dependent patients during a program of alcohol rehabilitation.

A randomized double-blind trial was performed comparing two groups receiving daily either a combination of micronutrients (vitamin A 5000 IU, vitamin D_3 400 IU, vitamin E 15 mg, vitamin B_1 IP 5mg, vitamin B_2 IP 5mg, nicotinamide IP 45mg, D-panthenol IP 5mg, vitamin B_6 IP 2mg, vitamin C IP 75 mg, folic acid 1000mcg, vitamin B_{12} IP 5mcg, dibasic calcium phosphate IP 70 mg, copper sulphate BP 0.1mg, zinc sulphate monohydrate USP 28.7, potassium iodide IP 0.025 mg, light magnesium oxide IP 0.15 mg) or a non-supplemented group. 160 male alcohol-dependent patients, 25-45 years of age without severe liver disease, hospitalized for a 21-day rehabilitation program were included.

Serum malondialdehyde, α-tocopherol, β-carotene and vitamin C were measured in serum, initially and after supplementation. In the non-supplemented group, serum concentrations of malondialdehyde decreased significantly ($p<0.05$) and vitamin C significantly increased ($p<0.001$), whereas α-tocopherol and β-carotene concentrations were unaffected. At the end of the hospital stay, serum indicators were significantly improved in the supplemented group as compared to the non-supplemented group for α-tocopherol, β-carotene and serum malondialdehyde ($p<0.001$).

The results indicate that a short-term supplementation with physiological doses of antioxidant vitamins during alcohol rehabilitation clearly improves micronutrient status indicators, and reduces oxidative stress.

Key Words: Micronutrients; Malondialdehyde; α-Tocopherol; β-Carotene; Vitamin C; Alcohol rehabilitation

Introduction

Ethanol oxidation involves three main metabolic pathways localized in three different sub-cellular compartments of the liver cell: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cytosol; the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum, and the catalase system in both peroxisome and mitochondria. Several studies have shown that the first stable product of ethanol metabolism via aldehyde oxidase (AO), acetaldehyde, may play a role in ethanol-induced free radical injury.[1,2] The enzyme can use several electron acceptors, but molecular oxygen is the physiological oxidant that is divalently reduced to produce H_2O_2. However, a part of the oxygen is univalently reduced, generating superoxide anion radical (O^{2-}).[3]

Acute ethanol (via production of NADH) and chronic ethanol (induction of P-450IIE1 uncoupling) administration may increase microsomal generation of oxygen radicals (ethoxy, hydroxyethyl). Both acute and chronic ethanol intoxications can increase local production of reactive oxygen species. Iron mobilization from ferritin by

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ethanol-stimulated O^{2-} can supply reactive ^{-}OH (hydroxyl) radicals. A direct increase of membrane permeability induced by ethanol may cause an increased susceptibility to lipid peroxidation. The development of oxidative stress in the liver may contribute to the hepatotoxic action of alcohol.[4]

Chronic alcoholism is associated with a high risk of micronutrient deficiency;[5,6] it may affect nutrient intake, increase the need for specific nutrients (especially when ROS are generated in large amounts), and interfere with their absorption, storage and utilization. Micronutrients such as retinol, α-tocopherol, vitamin C and carotenoids are important factors implicated in the defense against oxidative injury, and a deficit in any one of these elements can result in functional impairment of the overall antioxidant system.[7-10] Previously, some investigators have observed lower α-tocopherol, vitamin C and carotenoid status in alcohol-dependent patients when compared to controls.[11,12] Therefore, this study was designed to explore whether such serum indicators were altered by supplementation with a combination of antioxidant micronutrients (vitamin A, vitamin C and vitamin E) at physiological doses during alcohol rehabilitation.

Materials and Methods

This study was carried out after getting clearance from the Institutional Ethical Review Committee of Grant Medical College & Sir JJ Groups of Hospitals, Byculla, Mumbai. Informed consent was obtained from all the subjects. A discrimination procedure was used to separate alcoholics from non-alcoholic patients having hepatic disease, using a combination of the most promising models: CAGE,[13-15] Michigan Alcohol Screening Test (MAST),[16,17] Alcohol Use Disorder Identification Test (AUDIT),[18,19] and Severity of Alcohol Use Disorder Data (SADD).[20] Patients between 25 and 45 years of age, willing to participate in the study, and with no history of undergoing long-term medical intervention for various reasons like cancer, diabetes, advanced alcohol liver disorder, acute respiratory distress (ARD), chronic renal failure (CRF), cardiovascular diseases (CVS), etc., were included in the study. Patients with acute psychotic states were also excluded. Alcoholic patients (n=160) attending the de-addiction center who met the inclusion criteria and gave their informed consent were included in the study. Exclusion criteria for patients and controls were as follows:

- Patients below 25 and above 45 years
- Patients undergoing long-term medical intervention for various reasons.
- Excessive smoking evaluated according to Fagerstrom test for nicotine dependence with score more than 15.[21-23]
- Substance abuse involving cannabis, nicotine, opium and other psychotropic substances.
- Patients taking vitamins, antioxidants or any other significant supplements.
- Immuno-compromised and acute infectious states.
- Patients with acute psychotic states, or patients unwilling to participate in study.

Abstinence and treatment compliance were checked by regular interviews. In addition, during the rehabilitation program, patients had to stay in the hospital department, and no alcohol beverage was allowed to enter the hospital area. In addition, the patients did not take supplements containing any of the studied micronutrients. During the hospital stay, four main meals were served (breakfast, lunch, evening snack and dinner). Food composition of these meals was calculated in accordance to the "Recommended Dietary Allowances for Indians".[24] All the patients underwent a complete medical examination and a biological screening at the entrance, and three weeks of rehabilitation. A detailed standardized interview aimed especially at drinking history, tobacco and drug consumption was performed.

Participants were randomly assigned to one of two treatment groups (n=80 in both non-supplemented and supplemented groups). The supplemented group members received one capsule per day for a period of 21 days, each capsule containing vitamin A 5000 IU, vitamin D_3 400 IU, vitamin E 15 mg, vitamin B1 IP 5mg, vitamin B_2 IP 5mg, nicotinamide IP 45mg, D-panthenol IP 5mg, vitamin B_6 IP 2mg, vitamin C IP 75 mg, folic acid 1000mcg, vitamin B_{12} IP 5mcg, dibasic calcium phosphate IP 70 mg, copper sulphate BP 0.1mg, zinc sulphate monohydrate USP 28.7 mg equivalent to 10.4 mg of elemental zinc, potassium iodide IP 0.025 mg, and light magnesium oxide IP 0.15 mg. The other group was not provided with supplements. In each group, 5 subjects were withdrawn from the trial before the end. All the withdrawals were due to non-compliance with the rehabilitation program, or with micronutrient treatment. Statistical analyses were performed on the non-supplemented group of 80 subjects, and supplemented group of 80 subjects (all males).

Vitamin and carotenoid status, and measurements of biological indices were determined at the baseline, and after 21 days of supplementation. Ten ml of venous blood samples from overnight fasting individuals were collected. The blood samples were collected in plain tubes, and serum was separated by centrifuging at 2500 rpm for 7 minutes at room temperature, and was used for estimation of serum gamma-glutamyl transferase (GGT), glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT), and beta carotene, vitamin E, vitamin C and malondialdehyde (MDA). Haemolysed and turbid samples were discarded. Blood samples collected in EDTA tubes were used for estimating MCV. All fine chemicals used in this study were from Sigma (St Louis, MO, USA), and other reagents were obtained from E-Merck, India Ltd.

The serum lipid peroxidation was estimated by thiobarbituric acid (TBA) reactivity.[25,26] Malondialdehyde (MDA) and end-products of fatty acid peroxidation react with TBA to form a coloured complex that has maximum absorbance at 532 nm. MDA values were calculated from the absorbance coefficient of MDA-TBA complex at 532 nm, 156,000 cm^{-1} mol^{-1}. Serum vitamin E was estimated by the method of Baker et al.[27] Vitamin E from serum was extracted into n-heptane, which reacts with ferric chloride, and reduces ferric to ferrous ions. Ferrous ions then form a red coloured complex with 2,2-dipyridyl, which was read at 520 nm. The method proposed by Kyaw was used to estimate serum vitamin C.[28] Vitamin C from serum reacts with phosphotungstate to give a blue colour that has maximum absorbance at 600 nm. All spectrophotometric readings were taken on Shimadzu UV-160A, UV-Visible Recording Spectrophotometer. All the samples were run in duplicate, differences were statistically assessed using student t-Test,[29] by using statistical software MINITAB; ONE-WAY ANOVA was applied. The results obtained were expressed as Mean ± standard deviation (SD). For all comparisons, a p value ≤ 0.05 was considered to be statistically significant.

Results

At baseline, the remaining subjects in the supplemented and in non-supplemented groups were similar in terms of age, alcohol intake, as well as anthropometric indices (**Table 1**), and biological characteristics: GGT, SGOT, SGPT activities and MCV (**Table 2**). After the 21-day rehabilitation program GGT, SGOT, SGPT activities and MCV significantly decreased (**Table 2**), whereas anthropometric indices significantly increased (**Table 1**). At entrance in the study, no significant difference was observed between supplemented and non-supplemented group for vitamin C, vitamin E, beta-carotene and serum MDA levels (**Table 2**). In non-supplemented group after the 21 days of rehabilitation, serum concentrations of vitamin E and beta carotene were unaltered, whereas serum vitamin C levels increased significantly ($p \leq 0.001$), and serum MDA levels decreased significantly ($p \leq 0.05$). At the end of the hospital stay, serum indicators significantly improved in the supplemented group for vitamin E, beta carotene and also MDA ($p \leq 0.001$).

Discussion

The alcohol abuser group was carefully controlled, and withdrawal was monitored. However, subjects were selected according to strict criteria, and biological markers of alcohol consumption (GGT and MCV), and hepatocellar injury (SGOT and SGPT) were measured. The levels of biological markers of alcohol consumption and hepatocellar injury at entrance (GGT, MCV, SGOT and SGPT) were higher in the group of alcohol-dependent patients. The enzyme markers of liver cell membrane disruption (SGOT and SGPT) normalized after three weeks of abstinence, contrary to GGT activity and MCV. Generally, in abstinent patients without liver disease, SGOT and SGPT activities return to normal after three weeks, GGT activity after six to eight weeks, and MCV after three months.[30]

It is well known that antioxidant status plays a critical role in the defense against oxidative stress. In this study, it was observed that there is a significant decrease in antioxidant status with respect to vitamin E, C and beta carotene, which may be due to increased demands of vitamin E due to enhanced oxidative stress. These observations are in agreement with that of others.[31-33] Dupont et al reported an 18% decrease in vitamin E concentration in alcoholic patients.[34] Alpha tocopherol can act as chain-breaking antioxidant to prevent lipid peroxidation. When this antioxidant defense is impaired, free radicals cause cell injury. Alpha tocopherol stops lipid peroxidation by trapping the free radicals. In this process, alpha tocopherol is converted to alpha tocopheroxyl radical. Vitamin C regenerates alpha tocopherol from alpha tocopheroxyl radical. Vitamin C may have an important role in regeneration of reduced form of vitamin E.[35-37] It is known that vitamin C defi-

Table 1 Physical Findings of Subjects Before and After 3 Weeks of Alcohol Rehabilitation (Supplement and Nil Supplement Group)[a]

Parameters	Groups	Before Rehabilitation	After Rehabilitation
Age (years)	Nil Supplement	37.28±5.3	
	Supplement	36.60±6.2	
Alcohol intake (g/day)	Nil Supplement	99.6±15.9	~
	Supplement	101.3±10.6	~
Anthropometric Index[@]	Nil Supplement	0.14±0.017	0.20±0.04***
	Supplement	0.13±0.013	0.22±0.05***

[a] Results are expressed as mean ± standard deviation.
*** p< 0.001; rehabilitation effect for both groups in ANOVA for repeated values.
Differences in change between Nil Supplement and Supplement groups were non-significant for all variables.
~ alcohol intake was absent during rehabilitation programme
[@] Age independent anthropometric indices = (Weight in kg x 100) / (Height in cm)^2 (Normal range = 0.15-0.16)

Table 2 Characteristics and Serum Concentrations of Malondialdehyde, Vitamin E, Beta Carotene and Vitamin C in Subjects Before and After 3 Weeks of Alcohol Rehabilitation (Nil Supplement and Supplement Group) [a]

Parameters	Groups	Before Rehabilitation	After Rehabilitation
GGT (11-50 U/l at 37°C)	Nil Supplement	188.92±7.9	46.88±8.3***
	Supplement	183.76±8.8	33.45±2.9***
MCV (82-98 fL)	Nil Supplement	115.3±3.9	93.4±4.1***
	Supplement	110.7±1.1	96.4±4.1***
SGOT (0-40 IU/L)	Nil Supplement	74.70±1.2	31.43±5.5***
	Supplement	76.10±1.3	29.65±7.2***
SGPT (0-40 IU/L)	Nil Supplement	54.55±0.87	23.88±1.4***
	Supplement	56.18±4.2	34.49±1.4***
MDA (nmol/ml)	Nil Supplement	4.12±0.24	1.13±0.35*
	Supplement	4.30±0.34	0.82±0.32***
Beta Carotene (mg/dl)	Nil Supplement	82.7±0.8	89.49±0.5[#]
	Supplement	86.7±0.6	116.57±0.6***
Vitamin E (mg/dl)	Nil Supplement	0.56±0.23	0.59±0.24[#]
	Supplement	0.43±0.14	1.16±0.19***
Vitamin C (mg/dl)	Nil Supplement	0.70±0.20	1.21±0.6***
	Supplement	0.75±0.2	1.25±0.2***

[a] Results are expressed as mean ± standard deviation.
* p< 0.05; rehabilitation effect for both groups in ANOVA for repeated values.
*** p< 0.001; rehabilitation effect for both groups in ANOVA for repeated values.
Differences in change between Nil Supplement and Supplement groups were non-significant for all variables.
[#] Non-significant difference of parameter on rehabilitation

ciency occurs in alcoholism due to primary malnutrition, or reduced dietary intake. Due to reduced concentration of vitamin C, alpha tocopherol may not be regenerated from alpha tocopheroxyl radical at the same rate at which the latter is produced. Hence the concentrations of alpha tocopherol are found to be reduced in alcohol dependent patients.[33,38,39] This suggests that oxidative stress continues in the case of chronic alcohol intoxication.

During the rehabilitation period of 21 days, it was observed in the non-supplemented group that there was an increase in serum levels of vitamin C. Moreover, serum alpha tocopherol and beta carotene levels were not affected, but serum MDA level decreased significantly at the end of hospital stay. After abstinence, it was observed that there was a statistically significant increase in the levels of serum beta carotene, alpha tocopherol and vitamin C, and a decrease in the levels of serum MDA concentration of alcohol-dependent supplemented group. This suggests a specific effect of withdrawal and micronutrient supplementation on serum antioxidant vitamins and lipid peroxidation.

At the end of the trial, it was observed that higher serum concentrations of vitamin C, alpha tocopherol, and beta carotene occurred in participants who received multisupplement during the 21-day rehabilitation, than in the non-supplemented group. Such improvement after supplementation with physiological amounts of vitamins, beta carotene and trace elements has been previously described in healthy individuals.[40] The role of alcohol in vitamin C status is complex, and probably involves behavioural as well as metabolic influence. Alcohol consumption may reduce the intake and bioavailability of vitamin C, and increase urinary excretion,[41-44] as well as the requirement due to increased free radical activity associated with ethanol metabolism.[45,46] During alcohol rehabilitation, inverse processes probably occur and could explain the large response to vitamin C intake.

In conclusion, the results indicate that short-term supplementation (21 days) with moderate doses of antioxidant vitamins such as vitamin E, vitamin C and beta carotene during an alcohol rehabilitation program clearly improves micronutrient status, and minimizes oxidative stress, i.e., lipid peroxidation.

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