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Research Article

POSSIBLE INVOLVEMENT OF PEROXIDATIVE STRESS AND ANTIOXIDANT LEVELS IN ALCOHOLIC AND NON-ALCOHOLIC HEALTHY SUBJECTS

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ABSTRACT

Objectives: Antioxidant levels vary from person-to-person depending on their degree and type of activity, food, exposure to psychological stress, and contaminated environment. In addition, those who are older, have specific medical conditions, take drugs, smoke, and consume alcohol, and are exposed to solar radiation may require more carotenoids and other antioxidants. This led us to compare the antioxidant levels within alcoholics and non-alcoholic healthy subjects.

Methods: Malondialdehyde (MDA) and level of some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), antioxidant Vitamin A, Vitamin C, and Vitamin E were estimated in the circulations of alcoholics and non-alcoholic healthy subjects.

Results: Significantly increased concentrations of plasma thiobarbituric acid reactive substances, and significantly lowered levels of SOD, CAT, GSH, and GSH-Px were observed in alcoholics and may be due to their increased utilization to scavenge lipids peroxides. Increased levels of lipid peroxidation may be due to excessive oxidative stress. The comparison between alcoholics and non-alcoholics revealed 24% increased MDA in alcoholic subjects. Enzymatic (SOD, CAT, and GSH-Px), metabolic (GSH), and nutrient antioxidants (Vitamin A, E, C, and β-carotene) were lower in alcoholic as compared to non-alcoholic subjects.

Conclusion: Regardless of commercial brand, alcohol raises oxidative stress. When drinking alcohol and smoking or environmental contamination are coupled, this stress is significantly greater.

Keywords: Alcoholics, Peroxidative stress, Malondialdehyde, Superoxide dismutase, Catalase, Glutathione, and Glutathione peroxidase.

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INTRODUCTION

Almost every organ in the body suffers damage from excessive alcohol consumption. As the liver is the principal location for alcohol metabolism, it must initially take the brunt of any alcohol-related harm [1,2]. In India, alcoholic liver disease (ALD) has grown to be a significant source of morbidity and mortality [3]. In India, excessive alcohol use is the leading cause of liver cirrhosis [4]. The most significant risk factor for the onset of ALD is the amount and kind of alcohol consumed [5]. About 90% of people with ALD who consume more than 60 g/day progress, whereas 5–15% of them who continue drinking develop alcoholic hepatitis [6-8].

The levels of antioxidants vary from individual to individual with the level and nature of activity, diet, and exposure to psychological stress and polluted environment. In addition persons who have certain medical conditions or taking medications, smoke and drink alcohol or are exposed to radiation may have increased requirements for carotenoids and other antioxidants (Gollnick *et al*, 1996) [9]. This prompted us to check the antioxidant levels in alcoholics and compare them with non-alcoholics.

METHODS

The study was conducted in a tertiary care hospital in Udaipur. 50 healthy non-alcoholic subjects and 50 alcoholic subjects were selected for this study. Detailed present and history of subjects were recorded on a separate pro forma regarding their general information, i.e., age, sex, height, weight, caste, religion, dietary habits, and education.

Biochemical estimations

Lipid peroxide was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by method of Buege [10] Superoxide dismutase (SOD) was assayed by the method of Mishra and Fridovich [11]. Catalase activity was assayed by the method of Sinha [12]. Glutathione peroxidase was estimated by method of Pierie [13] and Glutathione by Beutler E [14]. Vitamin A and β- carotene was estimated by Natelson [15]. Plasma vitamin C (ascorbic acid) was estimated by the method of Roe and Kuether [16]. Plasma vitamin E (α-tocopherol) was estimated by the method of Baker and Frank [17].

The data for biochemical analysis were expressed as mean and standard deviation. Statistical comparisons were performed by Student's "t" test.

RESULTS

Table 1 shows the comparison of alcoholics and non-alcoholics in the level of lipid peroxidation in plasma. Lipid peroxidation as assayed by TBARS level was significantly higher in alcoholics as compared with non-alcoholics subjects. Malondialdehyde (MDA) levels showed more rise (24%) in alcoholics.

Table 2 shows the level of antioxidants in the circulation of both groups with alcoholics and non-alcoholics. The enzymatic antioxidants such as SOD were 17% decreased in alcoholics as compared to non-alcoholics. Catalase in the hemolysate was about −8% higher in non-alcoholics as compared to alcoholics. Furthermore, the non-enzymatic antioxidants GSH were 11% decreased in alcoholics.

Table 1: Peroxidative stress in alcoholics and non-alcoholics

Parameters	Non-alcoholics (n=50) Mean±SD	Alcoholics (n=50) Mean±SD
MDA (nmol/mL)	3.10±0.71	4.11±4.20

MDA: Malondialdehyde, SD: Standard deviation

Table 2: Antioxidant levels in alcoholic and non-alcoholic healthy subjects

Parameters	Non-alcoholics (n=50) Mean±SD	Alcoholics (n=50) Mean±SD
SOD (U/mg Hb)	2.01±1.23	1.65±0.20
Catalase (μmol H_2O_2/min/mg protein)	87.21±13.07	80.11±16.11
GSH (mg/dL)	35.41±6.03	31.45±5.11
GSH-Px (mg GSH/min at 37°C)	2.18±1.03	1.58±1.23

SOD: Superoxide dismutase, GSH: Glutathione, GSH-Px: Glutathione peroxidase, SD: Standard deviation

Table 3: Vitamin antioxidant levels in alcoholic and non-alcoholic healthy subjects

Parameters	Non-alcoholics (n=50) Mean±SD	Alcoholics (n=50) Mean±SD
Retinol (μg/dL)	30.10±8.62	28.11±8.16
B-carotene (μg/dL)	130.53±46.12	129.15±38.17
Ascorbic acid (mg/dL)	1.26±0.28	0.71±0.17***
Tocopherols (mg/dL)	1.65±0.20	0.92±0.21***

***p<0.05, SD: Standard deviation

Table 3 shows the level of antioxidants and vitamins in alcoholics and non-alcoholics. Alcoholics showed lower levels of ascorbic acid, tocopherol, β-carotene, and retinol. The levels of Vitamin C and Vitamin E were 43% and 44% significantly decreased in alcoholics compared to non-alcoholics.

DISCUSSION

A comparison between alcoholic and non-alcoholic healthy controls revealed that alcoholics had depleted antioxidant levels except B-carotene. Fifty normal subjects were consuming alcohol every day. All of them belonged to the lower-middle economic group. They consumed country liquor which is quality-wise very poor weight liquor and is expected to have many other side effects as well. People usually feel shy to divulge the exact amount of consumption due to social constraints but from the information we could gather the consumption should be more than 100 g/day. This places them in the alcohol addict category as per the World Health Organization criteria. It is now well-recognized that alcohol causes multiple problems in human physiology besides social disadvantages.

Recent data indicate that it also behaves as a prooxidant, especially in the liver where it is metabolized. Our data support this contention. In our group, the mean MDA level was 4.11+0.20 which was significantly higher than non-alcoholic consuming group. [Table 1] shows that peroxide level may be due to unusual generation of superoxide anion or weak antioxidant defenses or both. We were able to assess the first cause but weaker antioxidant defenses battery was very much evident. Among nutrient antioxidants retinol, a-tocopherol, and ascorbic acid were significantly lower and so were the enzyme antioxidants SOD, catalase, and GSH. The deficit of nutrient antioxidants reflects a double disadvantage. First, their requirement becomes higher in alcoholics due to increased metabolic needs; and second, their lower level will decrease the scavenging capacity of the cell. Naturally, these alcohol addict -subjects were more vulnerable to oxidant injury due to their shortage. Aggravating this situation was the lower level of SOD which is the only enzyme responsible for dissimulation of superoxide anion. Naturally, its deficiency is likely to exacerbate pro-oxidant activity. The lower activity of both GSH-Px and catalase the decreased capacity for detoxification of H_2O_2, is expected to occur. The cumulative effect therefore could lead to oxidant insult. This is very much reflected in our data by raised MDA levels. Further, alcohol enlarges superoxide anion pool. The exact mechanism is not known but the one postulated by Lewis and Paton's (Feher *et al*., 1992) [18] is in the conversion of xanthine dehydrogenase to xanthine oxidase which increases superoxide anion generation. Thus, in our selected group of alcohol consumers, the oxidative offence may be for two reasons first increased superoxide anion and second, decreased antioxidant defenses.

Although clinically we did not notice any signs of liver injury our biochemical data do stress a possible adverse effect of oxidative insults at sub-clinical levels. Alcohol ingestion induces a cytochrome p-450 system which increases the production of acetaldehyde causing liver injury by forming adducts with protein, inactivating enzymes, damaging cell membranes, depleting GSH, and inducing free radical damage. Antioxidative protective mechanisms involve both enzymatic and non-enzymatic defense systems (Helmut and Norman, 1995) [19]. Impairments in such defense systems have been reported in alcoholics, including alterations of ascorbic acid (Lecomte *et al*., 1994) [20], α- tocopherol, and retinol (Bjørneboe, 1988) [21] also GSH, catalase (Jeffrey and Ronald, 1988) [22] and GSH-Px. These changes could be due to the direct effects of ethanol or the malnutrition associated with alcoholism.

Our results showed lower levels of Vitamin A than a non-alcoholic which is possible as absorption of vitamin is reduced in alcoholics (Leevy, 1970) [23]. Furthermore, our results mentioned that ethanol administration depresses Vitamin A levels. Furthermore, the antioxidant Vitamin C and Vitamin E are also decreased which was shown by Bjørneboe (1988) [21] and Hagen (1989) [24]. Vitamin E is a major antioxidant in the membrane and is viewed as the first line of defense against membrane lipid peroxidation (Ingold *et al*., 1987) [25] whereas Vitamin C acts as the primary antioxidant in the aqueous phase (Beyer, 1994 and Retsky *et al*., 1993) [26,27].

CONCLUSION

Alcohol is a significant financial and medical burden on the world and a growing contributor to chronic liver disease. However, there are no proven treatments for ALD at this time. ALD aetiology involves oxidative stress in a number of ways. So increase in antioxidant diet can help to reduce oxidative stress.

ETHICS CLEARANCE

The Institutional Ethics Committee provided the ethical clearance certificate.

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CONFLICTS OF INTEREST

No conflict of interest has been reported.

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