Original Paper

INCREASED SERUM HOMOCYSTEINE LEVELS AND GLUTATHIONE-S-TRANSFERASE ACTIVITIES IN ALCOHOLIC PATIENTS ATTENDING DE-ADDICTION CENTRE

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ПОВЫШЕННЫЙ УРОВЕНЬ СЫВОРОТКИ ГОМОЦИСТЕИНА И ДЕЙСТВИЯ ГЛУТАТИОНА-С-ТРАНСФЕРАЗЫ У ПАЦИЕНТОВ С АЛКОГОЛЬНОЙ ЗАВИСИМОСТЬЮ, ПОСЕЩАЮЩИХ НАРКОЛОГИЧЕСКИЙ ЦЕНТР

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The purpose of this study was to investigate the effect of heavy alcohol consumption on serum Malondialdehyde, homocysteine status and glutathione-S –transferase (GST) activities in alcoholics consuming illicit liquor from lower socioeconomic background attending deaddiction centre. The study was conducted in ninety alcoholic patients consuming illicit liquor from lower socio-economic background attending de-addiction centre and compared to healthy non alcoholic controls (n=90). Serum Malondialdehyde (MDA), serum homocysteine and activities of antioxidant enzyme glutathione-S – transferase (GST) were estimated. Alcoholics consuming illicit liquor attending de-addiction centre displayed significantly higher values of serum MDA concentration ($p<0.001$), serum homocysteine levels ($p<0.001$) and serum GST activities ($p<0.001$) as compared to their non alcoholic healthy controls belonging to the same socioeconomic background. Our results indicate that increase in serum Malondialdehyde (MDA) concentration marker of oxidative stress, serum homocysteine levels and serum glutathione-S-transferase (GST) activities may enhance the susceptibility to vascular diseases in heavy illicit drinkers with poor nutritional status.

Key words: homocysteine, glutathione-S-transferase, alcohol.

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Изучался эффект от интенсивного употребления алкоголя на сыворотку малондиалдегида, состояние гомоцистеина и активность глутатиона-с-трансферазы у алкоголиков из низкого социально-экономического слоя населения, употреблявших запрещенные (несертифицированные) спиртные напитки и посещавших наркологический центр.

В исследовании участвовали 90 пациентов с алкогольной зависимостью, употреблявших запрещенные спиртные напитки и посещавших наркологический центр, и контрольная группа – 90 лиц без алкогольной зависимости. Были оценены сыворотка малондиалдегида, сыворотка гомоцистеина и активность антиоксидант-фермента глутатиона-с-трансферазы.

У алкоголиков, употреблявших запрещенные спиртные напитки и посещавших наркологический центр, выявлен значительно более высокий показатель концентрации сыворотки малондиалдегида ($p<0.001$), уровня сыворотки гомоцистеина ($p<0.001$) и глутатиона-с-трансферазы ($p<0.001$) в сравнении с контрольной группой, принадлежащей к такому же социально-экономическому слою населения.

Результаты исследования показали, что с увеличением концентрации сыворотки малондиалдегида, сыворотки гомоцистеина и активности антиоксидант-фермента глутатиона-с-трансферазы может усиливаться восприимчивость к сосудистым заболеваниям при интенсивном употреблении запрещенных спиртных напитков и при недостаточном, неполноценном питании.

Ключевые слова: гомоцистеин, глутатион-с-трансфераза, алкоголь.

Introduction. Alcoholic beverages have been used and abused since the dawn of the history although most people who choose to drink can limit their intake to a level that produces no harm to their health or to society, about 34 percent of the population drinks approximately 62 percent of all alcoholic beverages consumed. This chronic heavy drinking is a significant factor in the development of alcohol dependence, or alcoholism, and is associated with serious adverse health consequences, including negative effects on the cardiovascular system, such as heart muscle disorders (i.e., cardiomyopathy), heartbeat rhythm irregularities (i.e., arrhythmias), high blood pressure (i.e., hypertension), and strokes [1].

Many epidemiologic studies have shown that alcohol consumption and the risk of cardiovascular disease are associated in a J-shaped fashion [2, 3]. Moderate drinkers of alcoholic beverages have a slightly reduced risk, whereas heavy drinking is associated with an increased risk [4]. The concentration of total plasma homocysteine (tHcy) also was suggested as an explanatory factor [5, 6]. The concentration of tHcy is a well-established indicator

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for the risk of cardiovascular disease [7, 8], and it seems to be related to alcohol consumption [9]. The nature of this relation is, however, not fully clarified. Studies performed among alcoholics showed that a chronic intake of alcohol leads to increased tHcy concentrations [10].

Homocysteine is sulphur containing amino acid formed during methionine metabolism [11]. It can dimerise to homocysteine, or form disulphide bonds with proteins to form so-called 'protein-bound' homocysteine. Homocysteine is a four-carbon amino acid [HS (CH2)2CHNH2COOH], resulting from the demethylation of methionine. Homocysteine is a dimer composed of two oxidized molecules of homocysteine linked by a disulfide bond. Multiple forms of homocysteine circulate in blood: the majority (65%) is disulfide linked to protein; ~30% is in an oxidized state, mostly as disulfide links to itself or cysteine; and ~1.5–4% is free reduced form. Storage of plasma or serum causes redistribution of these forms with an increase in the protein-bound fraction [12]. Increase in plasma concentration of homocysteine is common in patients with stroke, peripheral vascular disease [13], and coronary disease [14] and confer an independent risk of atherosclerosis [15]. Measurement of total plasma or serum homocysteine represents the sum of oxidized and protein bound homocysteine. Homocysteine contains a reactive sulfydryl group that can react with plasma constituents and this may promote oxidative damage. An elevated homocysteine level therefore induces thrombogenicity, causes procoagulant state and promotes the proliferation of smooth muscle cells [16].

There appears to be increasing evidence that alcohol toxicity may be associated with increased oxidative stress and free radical associated injury [17]. Oxidative damage induced by reactive oxygen species is caused by increased production of Superoxide anions (O^{2-}) and its metabolites and/or by reduced bioavailability of antioxidant defense. Alcohol is known to induce hyperlipidemia leading to enhanced lipid peroxidation [18]. Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues [19]. The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies [20]. Moreover the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions [21] and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated [22]. They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes.

Mammalian cells express a number of enzyme systems to detoxify ROS and their by-products, including glutathione-S-transferase (GST). GST is a cytoplasmic class of large family of enzymes with their maximal activity seen in the hepatocytes. GST's are believed to exert a critical role in cellular protection against ROS. Within the hepatocytes, GST's are involved in conjugating reduced glutathione to electrophiles, hydroperoxides and xenobiotics derived from the metabolism of ethanol [23].

In the present study, the following parameters were assessed in the serum to elucidate the oxidant antioxidant status in alcoholic patients consuming illicit liquor from low socio-economic background attending de-addiction centre. Serum Malondialdehyde (MDA) levels were measured as thiobarbituric acid reacting substances (TBARS), which serves as an index of extent of lipid peroxidation. The activities of antioxidant enzyme like serum glutathione-S-transferase (GST) and serum homocysteine levels were estimated. GST is an enzyme involved in antioxidant defense mechanism which combats oxidative stress and also involved in detoxication process. The present work is an attempt to determine the changes in oxidant – antioxidant status and its contribution to the risk of cardiovascular disease in alcoholism.

Methods. This study was carried out after getting clearance from Institutional Ethical Review Committee, Grant Medical College & Sir J .J. Groups of Hospitals, Byculla, Mumbai.

In the present investigation, attempts were made to design a discrimination procedure to separate alcoholics from controls and patients with non-alcoholic hepatic diseases using a combination of the most promising test. The most powerful discrimination model was constructed with the batteries of screening instruments for detecting alcohol problems. CAGE [24], Michigan Alcohol Screening Test (MAST) [25], Alcohol Use Disorder Identification Test (AUDIT) [26] and Severity of Alcohol Use Disorder Data (SADD) [27]. 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, Advance alcohol liver disorder, Acute Respiratory Distress (ARD), Chronic Renal Failure (CRF) and other Cardio Vascular Disease (CVS) serious medical, surgical, neurological conditions were included in the study. Also, patients with acute Psychotic state were excluded. Alcoholic patients (n=90) attending the deaddiction center who met the following inclusion criteria's and gave their informed consent were included in the study, These patients were matched for age, sex and socio economic status with normal controls (n=90) who were participating in a screening programme. These controls were, to their knowledge healthy and had no reason to consult their local doctors during the preceding 12 months. Further their Nutritional anthropometry (age independent anthropometric indices) was evaluated by the method of Rao`s [28].

Exclusion criteria for patients and controls are:

1. Patients below 25 and above 45 are excluded from the study, patients undergoing long term medical intervention for various reasons like Cancer, Diabetes, Advance alcohol liver disorder, ARD, CRF and other CVS serious medical, surgical, neurological conditions are excluded from the study.

2. Excessive smoking evaluated according to Fagerstrom test for Nicotine dependence with score more than 15 are excluded [29]. Substance abuse such as Cannabis, nicotine, opium and other psychotropic substances are excluded from the study.

3. Patients taking Vitamins and antioxidants or any other significant supplements.

4. Immunocompramise and acute infectious state.

5. Patients with acute Psychotic state or patients unwilling to participate in study.

A dietary survey of study population was conducted by oral questionnaire method to assess per day consumption of calories, fats and protein. The daily food intake was recorded on a presented proforma and the values were computed from standard chart of «Recommended Dietary Allowances for Indians» [30] and by estimating dietary antioxidant vitamins in the blood of study population. Further assessment of their Socio econom-

ic status was done with the help of personal interview based on per capita income and education [31].

Within 24 hours of admission & overnight fasting conditions a total of 10ml of venous blood samples were collected. From blood samples collected in plain tubes serum was separated by centrifuging at 2500 rpm for 7 minutes at room temperature and were used for estimation of Serum Total Bilirubin , γ-Glutamyl Transferase (GGT), Serum glutamic-oxalacetic transaminases (SGOT), Serum glutamic-pyruvic transaminases (SGPT), Serum Malondialdehyde (MDA), Serum Homocysteine and Serum GST. Hemolytic or turbid samples were discarded. Blood sample collected in EDTA tube 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. Total alcohol content in the liquor samples collected from the patients were analyzed by Gas Chromatography [32].

The serum lipid peroxidation was estimated by thiobarbituric acid (TBA) reactivity [33, 34]. Malondialdehyde (MDA) and end product of fatty acid peroxidation, reacts with TBA to form a colored 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 Glutathione-S-Transferases (GST) activity was measured by the method of Habig William et al. [35], Homocysteine in serum was measured by using EIA kit method manufactured by Bio-Rad.

All spectrophotometric reading were taken on Shimadzu UV-160A, UV-Visible Recording Spectrophotometer. All the samples were run in duplicate, and were statistically assessed using student t-Test [36], by using statistical software MINITAB, were ONE-WAY ANOVA is being applied. The results obtained were expressed as Mean ± Standard deviation (SD).

Results. Our findings based on usage of traditional markers like Total bilirubin, (GGT) γ-Glutamyl Transferase, (MCV) Mean corpuscular volume, SGOT and SGPT, in serum to detect heavy drinking showed significantly increased values as compared to the control group [p<0.001] (Table 1). There was also statistically significant increase in the levels of serum MDA, serum homocysteine and serum GST activities in alcoholic patients compared to controls [p<0.001] (Table 2).

Discussion. In our study the alcohol dependent population consuming illicit liquor belongs to lower socioeconomic group on their personal interview scores based on modified socio economic status scale Kuppuswamy et al. [31], they live in slum or skid road side in metropolitan city like Mumbai. They are averse to hardworking, illiterate and live in poverty; they are careless and ignorant about their health, hygiene and nutrition. This population, which resides in slum areas for example dharavi in our study population are very fond of liquor, they have penchant for locally made liquor known as “Hath Bhatti”. This selected alcoholic population is grossly undernourished compared with their age, sex matched non-alcoholic control. It will be worthwhile to mention that in these poor communities with severe alcohol dependence the inadequate diet is due to financial constraints, as all their earnings are utilized in alcohol intoxication and gambling, all these factors should have multifarious adverse effects on health including oxidant/antioxidant balance. This is presently recognized as one of the important determinants of ageing process and numerous diseases. To the best of our knowledge the present study is a maiden effort to evaluate the effect of chronic alcohol intake of illicit liquor in this alcoholic populations belonging to lower socio- economic background with poor daily nutrient intake of dense nutrients.

In the present study the lipid peroxidation product i.e. MDA levels have been increased significantly in serum of the alcoholic patients consuming illicit liquor from low socio- economic background compared to controls (Table. no.2). Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. These results are in good agreement and run consistently well with the previous reports by Matsurma et al. [37], Suematsu et al. [38], Naveau et al. [39], Dupont et al. [40], Paramahamsa et al. [41], Ucar et al. [42] and Das et al. [43],

Table 1

Liver function tests in normal controls and alcoholics

Liver function test	Normal controls (n=90)	Alcoholics (n=90)
Total Bilirubin (mg/dl)	0.76 ± 0.44	5.70 ± 3.89*
GGT(11-50 U/l for men)	16.53 ± 7.8	170.25 ± 7.6*
SGOT(0-40 IU/L)	25.33 ± 2.5	88.60 ± 3.2*
SGPT(0-40 IU/L)	20.44 ± 5.6	70.67 ± 7.9*
MCV(82-98 FL)	86.39 ± 3.0	161.40 ± 3.8*

Note: Results are expressed as mean ± standard deviation. * – differences between Controls and Alcoholics were significant for all biochemical parameters (p<0.001).

Table 2

Serum MDA, Homocysteine and GST in normal controls and alcoholics

Parameters	Normal controls (n=90)	Alcoholics (n=90)
Serum Malondialdehyde (nmol/ml)	2.13±3.05	9.4±3.6 *
Serum Homocysteine (μmol/l)	9.8±0.7	26.3±1.6 *
Serum GST (nmol CDNB conjugate formed/min/mg protein)	3.05±1.02	9.52±1.24 *

Note: Results are expressed as mean±standard deviation. * – differences between Controls and Alcoholic Patients were significant for all biochemical parameters (p<0.001).

which states that both serum and erythrocyte TBARS content was increased significantly in alcoholic patients as compared to their controls.Paramahamsa et al. [44], found erythrocyte lipid peroxidation was 4-fold and 6 - fold higher in alcoholic diabetics, compared to the control group, similarly Mottaran et al [45] states that the fighters of anti-MDA antibodies were significantly higher in heavy drinkers than in controls.

The Glutathione-S-Transferase is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals & the hepatic removal of potentially harmful hydrophobic compounds from blood [46]. We have observed a significant increase in the GST activity in our study population belonging to lower socioeconomic status and consuming illicit liquor compared to their respective controls (Table.2), the rise in the activity of GST could be due to its induction to counter the effect against increased oxidative stress. Our findings showed resemblance with the result obtained by Das et al. [43] have encountered rise in erythrocyte GST activity in alcoholic liver disease patients with both moderate and high alcohol intake. A few investigators in their animal trials stated that ethanol treatment to rats caused no change in hepatic GST activity as compared with those in control Balkan et al. [47], while investigators like Chen, et al. [48] reported GST activity was significantly increased in mice liver tissue, when treated with ethanol compared with controls.

Homocysteine has been recognized recently as a risk factor for vascular diseases. In our study, Serum Homocysteine levels were significantly increased in alcoholic patients consuming illicit liquor .An increased serum homocysteine level is associated with the formation of atherosclerotic plaques and myocardial infarction. The sulfhydryl groups in homocysteine were oxidized to disulfide catalyzed by the transition metals by which several reactive oxygen species and hydroperoxides were produced and initiates lipid peroxidation which is responsible for endothelial injury. It is well known that tHcy concentrations are greatly elevated in persons with chronic alcoholism [49, 50]. This effect is in part due to nutritional deficiencies of folic acid and vitamin B-6 in alcoholics. On the other hand, it was shown that chronic ethanol feeding inhibited methionine synthase in rats [51]. There is probably a direct interference of alcohol or its metabolites with the intracellular metabolism of folic acid, vitamin B-6, and vitamin B-12 at more than one site [49].

We postulate that the observed hyperhomocystenianemia in these alcoholic patients is partly due to their decrease intracellular folate levels. In addition, it has been known that ethanol has an effect on folate metabolism, which cannot be explained by an alcohol induced low intake of folate [52]. The aethiology of folate deficiency in alcoholism can be ascribed to several causes, such as low dietary intake, poor absorption, decrease hepatic uptake and retention, and increased urinary excretion of folate [53, 54]. Also elevated homocysteine levels may occur as a result of inherited disorder and/or non genetic factor that alter enzyme activity in the transsulphuration and remethylation pathways [55, 56].

Conclusion. In summary, there is growing evidence that chronic alcoholism is associated with oxidative damage and with a derangement in endogenous antioxidant system which combats oxidative stress, also sulphur amino acid metabolism. Ethanol induced hyperhomocystenianemia may mediate variety of symptoms which are observed in alcoholic patients. Hyperhomocystenianemia is a treatable condition considering the folate therapy may reduce the serum homocysteine levels [57-59]. This study reports the significance of assessing serum MDA, GST in order to detect the oxidative damage and antioxidant status in alcoholics consuming illicit liquor, also the detection serum homocysteine in order to detect the methylation deficiency in this patient with chronic illicit liquor intoxication. Nevertheless, further investigations are needed in this Indian population especially from low socio economic background consuming illicit liquor with poor nutritional status to clarify the role of homocysteine.

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