Background: Alcohol remains the single most significant cause of liver disease throughout the Western world, responsible for between 40 and 80% of cases of cirrhosis in different countries. Material & Methods: 164 alcoholic hepatitis patients were subjected to detailed clinical examination and laboratory investigations and the results were compared with 82 controls. Blood samples were collected for oxidative stress parameters. It was observed that there was a significant increase in activities of Catalase, SOD, MDA, GPX and GR activity in patients with alcoholic hepatitis when compared to controls. Results: Results of our study show higher oxygen free radical production, evidenced by elevated levels of MDA and decreased levels of Catalase, SOD, GPx, GR, and TAS activity, supporting the evidence of oxidative stress in alcoholic hepatitis patients. Decreased concentrations of antioxidant support the hypothesis that alcoholic hepatitis is an important causative factor in pathogenesis of lipid peroxidation. Conclusion: The antioxidant defense mechanisms might be impaired in patients with alcoholic hepatitis. These findings also provide a theoretical basis for development of novel therapeutic strategies, such as antioxidant supplementation. Keywords: Alcoholic Hepatitis, Antioxidant status

INTRODUCTION

Alcohol induced Alcoholic Hepatitis disease with genetic, psycho-social and environmental factors influencing its development and manifestations. The alcoholic hepatitis is considered to be a major cause of morbidity and mortality. In recent years, oxidative stress has been implicated in the path physiology of a large number of disease or disorders which are initiated and/or exacerbated by pro-oxidants such as various drugs including alcohol and food additives. Besides, ingested alcohol produces striking metabolic imbalances in the liver. It leads to the formation of reactive oxygen species (ROS). Inadequate removal of ROS may cause cell damage by attacking membrane lipids, proteins and inactivating enzymes thus mediating several forms of tissue damage [1]. At present, except for the abstinence of alcohol abuse, there is no effective modality of either prevention or treatment. We have taken 24 hr cycle blood sample in this research due to the concentration of antioxidant and oxidative stress markers is different in 24 hrs cycle. The importance of 24 hr cycle is not only in diagnosis but it also plays a very important role in optimizing the time of treatment.

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like regulate your sleep/wake patterns, make you a morning person or a night owl, control concentration, and give you your best physical coordination in late afternoon. These rhythms also produce longer body cycles, such as the female menstrual cycle and reproduction [5]. The incidence of Alcohol Hepatitis is increasing day by day specially in the developing countries including India. The present study was planned with the objectives to investigate the oxidative damage and the efficiency of antioxidant defense system in patients of alcoholic hepatitis in the socioeconomic belt of Srinagar, Garhwal, Uttarakhand.

MATERIALS AND METHODS

Study design: An observational analytical study
Ethical approval: This study was approved by Institutional Ethical Committee (IEC) of Santosh Medical College and Hospital, along with informed consent.
Study setting: The place of Research was Veer Chandra Singh Garhwal Govt. Institute of medical sciences, Srinagar Garhwal and Santosh Medical College & Hospital, Ghaziabad.
Study duration: Duration of this study was Jan 2013 to Dec 2016.
Sample size: sample size was n= 82 taken as controls and n=164 taken as alcoholic hepatitis patients.
Inclusion criteria: Men/Women 18-70 years of age and clinically stabilized Alcoholic hepatitis patients. In this study One sixty four clinically, pathologically proven fresh cases of alcoholic hepatitis (age: 21-45 years), only minimal and moderately advanced patients of alcoholic hepatitis were included. For comparison 82 clinically healthy volunteers of either sex (age: 17-40 years) were included as control group.
Exclusion criteria: Viral Hepatitis, patients with CNS disorders, Psychiatric patients, pregnant, lactating women and patients with Diabetes and hypertension.
Grouping: Group 1: Control n=82, Group 2: Alcoholic n=164.

Sample collection: All participants were synchronized for one week with diurnal activity from about 6:00 to about 22:00 hours and nocturnal rest. Six milliliters of blood was collected from each subject at fixed time points for one complete 24 hour cycle, at 06:00, 12:00, 18:00 and 00:00 hrs in plain and sterile vials containing heparin as anticoagulant.

Methodology: Malondialdehyde (MDA): The plasma was separated and analyzed for lipid peroxidation levels were measured by the thiobarbituric acid (TBA) reaction using the method of Ohkawa et al (1979) [5]. MDA levels were measured spectro-photometrically by the reaction of TBA with MDA at 532 nm.

Super Oxide Dismutase (SOD): The haemolysate was prepared from the red cells and used for the measurement of the activities of enzymes SOD assay was carried out by method described by McCord and Fridovich etal (1969) [6] which was measured at 560 nm.

Glutathione peroxidase (Gpx): Gpx estimation was done by method described by paglia and valentine (1976) [8], which was measured at 340 nm.

Glutathione reductase (GR): GR estimation was done by method described by Hazelton and Lang [9] (1985) which was measured at 340 nm.

Total antioxidant status (TAS): TAS was analyzed by random ABTS kit method ABTS* (2,2'- Azino-di-[3 ethylbenzthiazolinesulphonate]) [10] is incubated with a peroxidase (metmyoglobin) and H2O2to produce the radical cation ABTS. This has a relatively stable blue-green colour, which was measured at 600 nm.

Statistical analysis: Statistical analysis of data was done by using the statistical package for social sciences (SPSS 12) for windows Software, Microsoft excel 2007 and Scientific Calculator. Result were expressed as Mean±S.D.

RESULTS

Table 1. Antioxidants status of control and patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>6.00 (hrs)</th>
<th>12.00 (hrs)</th>
<th>18.00 (hrs)</th>
<th>00.00 (hrs)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (mmol/l)</td>
<td>Control</td>
<td>1.90±0.04</td>
<td>2.20±0.05</td>
<td>2.51±0.04</td>
<td>1.76±0.03</td>
<td>2.28±0.03</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Patients</td>
<td>1.92±0.04*</td>
<td>3.86±0.03</td>
<td>2.56±0.01</td>
<td>2.73±0.02</td>
<td>2.95±0.04</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>Control</td>
<td>20.12±0.03</td>
<td>19.54±0.02</td>
<td>18.43±0.04</td>
<td>18.65±0.05</td>
<td>19.95±0.05</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Patients</td>
<td>16.31±0.04</td>
<td>15.45±0.03</td>
<td>14.9±0.04</td>
<td>14.67±0.0*</td>
<td>15.89±0.04</td>
</tr>
<tr>
<td>Catalase (U/ml)</td>
<td>Control</td>
<td>15.41±0.06</td>
<td>14.92±0.03</td>
<td>13.39±0.05*</td>
<td>13.56±0.04</td>
<td>14.55±0.05</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Patients</td>
<td>12.59±0.05*</td>
<td>13±0.05</td>
<td>13±0.05</td>
<td>13±0.05</td>
<td>11.72±0.04</td>
</tr>
<tr>
<td>Gpx (IU/l)</td>
<td>Control</td>
<td>3.49±0.06</td>
<td>4.12±0.04</td>
<td>4.50±0.02</td>
<td>3.45±0.04</td>
<td>4.13±0.03</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Patients</td>
<td>3.05±0.05</td>
<td>3.00±0.01</td>
<td>3.19±0.04</td>
<td>3.33±0.05</td>
<td>3.23±0.02</td>
</tr>
<tr>
<td>GR (IU/l)</td>
<td>Control</td>
<td>5.90±0.02</td>
<td>5.01±0.05</td>
<td>4.01±0.03</td>
<td>3.97±0.04</td>
<td>5.03±0.04</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Patients</td>
<td>5.02±0.04</td>
<td>4.99±0.03</td>
<td>4.01±0.04</td>
<td>3.02±0.03</td>
<td>4.52±0.03</td>
</tr>
<tr>
<td>TAS (mmol/l)</td>
<td>Control</td>
<td>1.6±0.05</td>
<td>1.90±0.02</td>
<td>1.6±0.03</td>
<td>1.5±0.04*</td>
<td>1.85±0.03</td>
</tr>
<tr>
<td></td>
<td>Alcoholic Patients</td>
<td>1.2±0.03</td>
<td>1.3±0.04</td>
<td>1.4±0.02</td>
<td>1.1±0.05*</td>
<td>1.47±0.02</td>
</tr>
</tbody>
</table>

* P<0.001 * values are expressed as ± SD
A marked circadian variations in MDA concentration and activities of anti-oxidant enzymes SOD, CAT, GPx and GR and TAS were recorded in healthy Indians and patients suffering from alcoholic hepatitis in the present study. The plasma lipid peroxide levels were observed to be maximum at 18:00 and minimum at 06:00 with significant amplitude and acrophase around 16:21 in healthy volunteers. The circadian acrophase of plasma MDA occurred 3 hour later in alcoholic hepatitis patients with maximum concentrations at 12:00 and minimum at 00:00 with decrease in circadian amplitude and increase in MESOR. Moreover, plasma lipid peroxide levels were noted to be elevated at all-time points of 24-hours sleep-awake cycle in comparison to healthy controls. The increased plasma lipid peroxides in alcoholic hepatitis could probably be due to its direct involvement in the pathogenesis of the disease.

**DISCUSSION**

We have compare this results to the previous studies, we have found that Lipid peroxidation in cell membranes and subcellular organelles has been proposed as a primary mechanism for cellular membrane dysfunction and tissue injury associated with free-radical initiated processes. Present observations are in agreement with other such reports Shaw et al [10], the present investigator has not come across any such report regarding circadian nature of lipid peroxides in alcoholic hepatitis patients.

Elevated concentrations of lipid peroxides may disturb relations between protective and aggressive factors at the tissue and molecular level leading to hepatic damage. Although much is known about the chemistry of lipid peroxidation and cellular defense mechanisms, chronobiological studies are needed to quantify the various cellular components involved in these processes for better management, prognosis and treatment.

**CONCLUSIONS**

In this study we were compare the MDA concentration and activity of SOD, CAT, GPx, GR and TAS in control compare with patients of alcoholic hepatitis. A statistically significant circadian rhythm was recorded in SOD, CAT, GPx and GR concentration in clinically healthy subjects and alcoholic hepatitis patients. The present observation support the involvement of free radicals in Alcoholic hepatitis patients and the assessment of its circadian nature may prove to be important in the clinical evaluation of the disease stage and its better management. The present study observations are needed to correlate the lipid peroxide levels with free radical scavengers, its nature, status and rhythm after administration of known dietary and therapeutic antioxidants in such pathological situations and thereby opening new chapters in understanding the pathogenesis of the disease.

**Limitations:** The present study observations are needed to correlate the lipid peroxide levels with free radical scavengers, its nature, status and rhythm after administration of known dietary and therapeutic antioxidants in such pathological situations and thereby opening new chapters in understanding the pathogenesis of the disease.

**Conflict of interest:** Nil

**REFERENCES**


10. ABTS kit method ABTS® (2,2′-Azino-di-[3-ethylbenzthiazoline sulphonate]) random Kit method at 600 nm.


