Original Article

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Alteration in Biochemical and Antioxidant Parameters in Patients with Alcoholic Liver Injury

Biochemistry Section

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ABSTRACT

Introduction: Alcoholic Liver Disease (ALD) is a result of excessive consumption of alcohol for long duration. Although several biomarkers associated with liver status are known to be influenced by excessive consumption of alcohol, however, the effect of alcohol quantity on biochemical changes and oxidative stress in ALD patients has not explained very well in previous studies.

Aim: To find out the alteration in biochemical parameters related to liver functions, antioxidants levels and oxidative stress in ALD patients (both moderate and heavy drinkers).

Materials and Methods: In the present study, 260 subjects were recruited from the Department of Medicine, Gastroenterology, Sri Aurobindo Institute of Medical Sciences (SAIMS) and Hospital, Indore, India and divided in three groups: Group I-(n=75) diagnosed cases of ALD with moderate drinkers Group II-(n=92) diagnosed cases of ALD with heavy drinkers; and Group III included (n=93) normal healthy controls. The levels of serum enzymes such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), and γ-Glutamyl Transferase (GGT), total protein, total bilirubin and lipid parameters such as total cholesterol, triglyceride, High Density Cholesterol (HDL) and Low Density Cholesterol (LDL) were measured by fully automated chemistry analyser. The levels of antioxidants such as Superoxide Dismutase (SOD), Catalase (CAT) and oxidative stress marker such as Malondialdehyde (MDA) was estimated by spectrophotometric method. $p<0.05$ was considered as a significant level.

Results: The present study demonstrate that the serum levels of AST, ALT, ALP, GGT, total bilirubin total cholesterol, triglyceride, LDL, VLDL and plasma MDA were significantly increased ($p<0.001$) and serum total protein, blood levels of SOD and CAT activity were significantly decreased ($p<0.001$) in Group I (ALD with moderate drinkers) as well as Group II (ALD with heavy drinkers) when compared with Group III (healthy controls), respectively. On comparison of Group I (ALD with moderate drinkers) with Group II (ALD with heavy drinkers), a significant difference was found only for LDL cholesterol, serum total bilirubin and plasma MDA.

Conclusion: Alcohol-induced liver injury is linked to significant alteration in various biochemical parameters and oxidative stress as observed by reduced levels of antioxidants in moderate as well as in heavy drinkers.

Keywords: Alcoholic fatty liver disease, Malondialdehyde, Oxidative stress, Superoxide dismutase

INTRODUCTION

Excessive alcohol consumption damages nearly every organ in the body. Liver is the primary site for alcohol metabolism hence has to bear the brunt of alcoholic injury at the earliest [1,2]. ALD has become a major cause of morbidity and mortality in India [3]. Excess alcohol consumption is the most common reason for liver cirrhosis in India [4]. The quantity and the type of alcohol which is ingested is the most important risk factor for the development of ALD [5]. ALD progresses in about 90% of cases who consumes alcohol more than 60 gm/day however, 5 to 15% of them develop alcoholic hepatitis who continues drinking [6-8].

In recent years, conventional biochemical markers have provoked the interest of researchers to study the alcohol-mediated damages in liver. Besides the clinical history for chronic alcoholism, estimation of ALT and AST are predominant laboratory investigations in aminotransferases, reflecting the damage to hepatocytes [9]. GGT is a well-established serum biomarker of ALD and it exists in the plasma membrane of hepatocytes [10,11]. In the absence of bone disease or pregnancy, elevated levels of ALP activity usually reflect impaired biliary tract function. Slight to moderate increases in ALP occur in many patients with liver disorders such as hepatitis and cirrhosis [12-15]. More than 80% of patients with ALD shows AST: ALT ratio ≥2 and known as a valuable diagnostic marker for ALD [16,17]. Serum protein and bilirubin are also indicator tests of altered hepatic activity [18,19]. Alcohol causes alteration in various parameters of lipid metabolism therefore, it is useful to study the lipid profile also in patients of ALD to understand the effects of increasing levels of alcohol consumption [20].

As liver is the main organ responsible for metabolising alcohol in the body [21] therefore, the different pathways of ethanol metabolism have numerous consequences that contribute to the tissue damage in alcoholic patients. These consequences resulting in the formation of adducts and highly reactive oxygen-containing molecules such as Reactive Oxygen Species (ROS) production that can lead to oxidative stress induced liver injury [22]. Ethanol metabolism by the support of Electron Transport Chain (ETC) produces ROS that is responsible for lipid peroxidation. The lipid peroxidation process results in the formation of a compound known as MDA [23]. MDA is a known marker of the oxidative stress-mediated lipid peroxidation [4]. Overconsumption of alcohol enhanced oxidative stress which is responsible for development of ALD [24].

Antioxidants are the scavenger of free radicals and inhibit the oxidation of lipids and proteins. Reduced activity of antioxidants promotes cell damage [25]. Alcohol-mediated higher production of pro-oxidants as well as reduced activity of antioxidant system may contribute to the development of ALD [5]. An experimental study has reported lower activity of SOD and CAT in heavy alcohol group [26].

In general, it is noted that the severity of ALD depends on quantity of alcohol, pattern of alcohol and duration of alcohol consumption [27]. Despite this, very few studies have been reported the effect of alcohol quantity on biochemical changes and oxidative stress parameters in ALD patients [28,29].

The present study was designed to measure the biochemical parameters such as ALT, AST, ALP, GGT, total protein and total bilirubin related to liver function, lipid profile parameters such as total cholesterol, triglyceride, HDL, LDL, antioxidants such as SOD, CAT and oxidative stress parameter as MDA in patient with ALD with moderate and heavy drinkers.

MATERIALS AND METHODS

The present case-control cross-sectional study was carried out in the Department of Biochemistry from May 2016 to April 2018. The study subjects were recruited from Department of Medicine, Gastroenterology Clinic at SAIMS College and Hospital, Indore, India. The study was approved by the Institutional Ethical Committee of SAIMS College and PGI Institute, Indore and ethical clearance number were SAMC/SS-02; Dated-26.02.2016. Patients were recruited for the study after taking their written informed consent. The sample size was determined by considering the prevalence rate of ALD in India as reported by previous study [4]. A total of 260 subjects were enrolled for the study and divided into three groups: Group I- 75 (45 males and 30 females) diagnosed cases of ALD with moderate drinkers; Group II- 92 (57 males and 35 females) diagnosed cases of ALD with heavy drinkers; and Group III- 93 (60 males and 33 females) normal healthy controls without any habit of alcohol drinking. The age was between 30 to 60 years for both genders. The subjects having diabetes, obesity, essential hypertension, thyroid disease, nephritic disease, cardiovascular associated liver disease, malignancy, asthma, gout, hyperlipidemias, other infectious diseases and pregnant women were excluded from the study. The alcoholic patients were classified as per the Paton criteria [30] into two categories. According to this criteria, alcoholic subjects were classified in two groups: (a) high alcohol intake group (heavy drinkers) was defined when the study subjects had been drinking >80 gm alcohol per day at lest last one year; and (b) moderate alcohol intake group (moderate drinkers) was defined when the study subjects had been drinking <80 gm alcohol per day.

Sample Collection and Analysis of Biochemical Parameters

Blood sample was collected after 10-12 hour fasting state. The analysis of AST, ALT, and ALP, GGT, total bilirubin, total protein and lipid profile parameters were carried out by fully automated chemistry analyser in clinical lab at SAIMS, Indore. Estimation of blood catalase activity was carried out by Aebi (1983) method [31]. Determination of SOD was done by Marklund S and Marklund G (1974) method [32]. Plasma MDA was measured by spectrophotometric method [33].

STATISTICAL ANALYSIS

SPSS version 20.0 was used for the statistical analysis. Data were presented as Means±Standard Deviation (Means±SD). Student's t-test was used for comparison of data in two groups. p<0.05 was considered as significant level.

RESULTS

Age distribution of study groups: Out of 260 subjects, 75 were found as moderate drinker and 92 found as heavy drinker According to age distribution, 41 to 50 years age group subjects were in higher numbers as compared to other age groups [Table/Fig-1].

Age (year)	ALD with heavy drinkers (n=92)	ALD with moderate drinkers (n=75)	Healthy controls (n=93)
30-40	18	7	20
41-50	43	41	39
51-60	31	27	34

[Table/Fig-1]: Age distribution of ALD patients and healthy controls.
n: Number

Anthropometric characteristic of ALD with moderate drinkers and heavy drinkers: The BMI was found significantly (p<0.001) increased in ALD with moderate drinkers as compared to healthy controls. Also, the BMI was found significantly (p<0.001) increased in ALD with heavy drinkers as compared to healthy controls. A significant difference was found for BMI on comparison between ALD with moderate drinkers and heavy drinkers [Table/Fig-2].

Liver profile in ALD with moderate drinkers and heavy drinkers: The AST, ALT, ALP, GGT, and total bilirubin were found significantly (p<0.001) increased and a significant (p<0.001) decreased level of total protein was found in ALD with moderate drinkers as well as ALD with heavy drinkers when both groups were compared to healthy controls. No significant difference was observed in liver profile parameters on comparison between moderate drinkers and heavy drinkers, except serum total bilirubin [Table/Fig-3].

Lipid profile in ALD with moderate and heavy drinkers: The serum total cholesterol, triglyceride, LDL-C and VLDL-C levels were found significantly (p<0.001) increased and no significant (p<0.79) difference of HDL-C found in ALD with moderate drinkers as compared to healthy controls. Similar, the serum total cholesterol, triglyceride, LDL-C and VLDL-C levels were found significantly (p<0.001) increased and no significant (p<0.40) difference of HDL-C was found in ALD with heavy drinkers as compared to healthy controls. On comparison of moderate drinkers and heavy drinkers, no significant result was found for lipid profile parameters however, serum LDL cholesterol was found significant on this comparison of these groups [Table/Fig-4].

Parameter (s)	ALD with moderate drinkers (n=75)	ALD with heavy drinkers (n=92)	Healthy controls (n=93)	*p-value	#p-value	$p-value
Height (m)	1.68±0.09	1.68±0.11	1.70±0.10	0.094	0.601	1.000
Weight (kg)	68.08±7.80	70.93±8.95	66.64±7.99	0.720	0.320	0.02
BMI (kg/m^2)	21.60±1.20	20.40±2.1	24.60±2.20	0.001	0.001	0.001

[Table/Fig-2]: Anthropometric characteristics of ALD with moderate and heavy drinkers.
Data are presented as Mean±SD; n: Number; BMI: Body mass index; *Comparison between healthy controls and ALD with moderate drinkers; #Comparison between healthy controls and ALD with heavy drinkers; $Comparison between ALD with moderate drinkers and heavy drinkers, p<0.05 was considered as significant level

Parameter (s)	ALD with moderate drinkers (n=75)	ALD with heavy drinkers (n=92)	Healthy controls (n=93)	*p-value	#p-value	$p-value
SGPT (IU/L)	119.16±33.03	124.22±35.95	28.85±8.49	0.001	0.001	0.101
SGOT (IU/L)	110.93±35.14	116.18±30.73	27.65±7.40	0.001	0.001	0.304
GGT (IU/L)	101.85±40.04	106.63±51.83	24.67±9.82	0.001	0.001	0.888
ALP (IU/L)	189.01±45.13	197.07±44.18	86.10±24.47	0.001	0.001	0.247
Total protein (gm/dL)	4.71±0.92	4.85±0.98	6.99±0.22	0.001	0.001	0.207
Total bilirubin (mg/dL)	1.51±0.24	1.79±0.14	0.61±0.15	0.001	0.001	0.001

[Table/Fig-3]: Liver function tests in ALD with moderate and heavy drinkers.
Data are presented as Mean±SD; n=number; *Comparison between healthy controls and ALD with moderate drinkers; #Comparison between healthy controls and ALD with heavy drinkers; $Comparison between ALD with moderate drinkers and heavy drinkers; p<0.05 was considered as significant level

Parameter (s)	ALD with moderate drinkers (n=75)	ALD with heavy drinkers (n=92)	Healthy controls (n=93)	*p-value	#p-value	$p-value
Total cholesterol (mg/dL)	248.13±31.88	259.85±39.83	118.19±20.48	0.001	0.001	0.054
Triglyceride (mg/dL)	189.69±38.46	189.20±46.18	123.58±13.90	0.001	0.001	0.941
LDL-C (mg/dL)	169.36±34.03	181.02±38.74	113.86±18.33	0.001	0.001	0.042
HDL-C (mg/dL)	35.01±3.03	36.04±3.71	40.01±7.68	0.790	0.403	0.547
VLDL-C (mg/dL)	38.38±7.97	37.89±7.13	24.72±2.82	0.001	0.001	0.675

[Table/Fig-4]: Lipid profile in ALD with moderate and heavy drinkers.
Data are presented as Mean±SD; n: Number; *Comparison between healthy controls and ALD with moderate drinkers; #Comparison between healthy controls and ALD with heavy drinkers; $Comparison between ALD with moderate drinkers and heavy drinkers, p<0.05 was considered as significant level

Parameter (s)	ALD with moderate drinkers (n=75)	ALD with heavy drinkers (n=92)	Healthy controls (n=93)	*p-value	#p-value	$p-value
Blood SOD (U/g of Hb)	2.59±1.13	2.66±1.03	5.67±1.06	0.001	0.001	0.674
Blood CAT (U/g of Hb)	2.57±0.30	3.04±1.23	6.72±1.07	0.001	0.001	0.798
MDA (µmol/mL)	7.35±2.66	8.17±2.25	2.80±0.52	0.001	0.001	0.032

[Table/Fig-5]: Blood activity of SOD, CAT and plasma MDA levels in ALD with moderate and heavy drinkers.
Data are presented as Mean±SD; n: Number; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde; *Comparison between healthy controls and ALD with moderate drinkers; #Comparison between healthy controls and ALD with heavy drinkers; $Comparison between ALD with moderate drinkers and heavy drinkers; p<0.05 was considered as significant level

Blood activity of SOD, CAT and plasma MDA levels in ALD with moderate and heavy drinkers: The plasma MDA level was significantly (p<0.001) increased and blood activity of SOD and CAT were significantly (p<0.001) decreased in ALD with moderate drinkers as compared to healthy controls. The plasma MDA level was significantly (p<0.001) increased and blood activity of SOD and CAT were significantly (p<0.001) decreased in ALD with heavy drinkers as compared to healthy controls. However, no significant difference was found for antioxidant parameters on comparison between moderate drinkers and heavy drinkers but significant difference was observed for plasma MDA in both groups [Table/Fig-5].

DISCUSSION

ALD is one of the leading causes of morbidity and mortality associated with alcohol consumption in India. In the present study according to age distribution, the ALD subjects were in higher numbers in the age group of 41 to 50 years. Our result was closely related with study done by Patil AM et al., [4]. ALD with heavy drinkers had significant low body weight, low BMI as compared to healthy controls in the present study. Addolorato G et al., has also reported a lower body weight in alcoholics as compared to social drinkers due to reduction of fat mass [34]. In another study of World MJ et al., has also shown that reduced body weight was the best clinical indicator of alcohol abuse [35]. In contrast to the present result Vozzo CF et al., have reported that elevated BMI is a risk factor in ALD as well as Non-Alcoholic Fatty Liver Disease (NAFLD) [36]. The important finding of the present study is a significant difference noted in BMI on comparison of moderate and heavy drinkers.

In the present study, significantly higher levels of ALT, AST, ALP, and GGT were observed in ALD with heavy and moderate drinkers in comparison to healthy controls. There are higher enzyme activities in moderate drinkers compare to healthy controls (occurrence of early biochemical changes in response to ethanol intake). Alatalo P et al., have reported that serum GGT, AST, ALT were significantly higher in heavy drinkers in comparison to moderate drinkers in their study [37]. In this study patients with ALD showed hyperbilirubinemia and the significantly increased levels of total bilirubin were found in both heavy as well as moderate drinkers with ALD. In support of the present result, Das SK and Vasudevan DM, also found elevated serum bilirubin level in heavy and moderate ALD patients [38]. In the present study, significant decrease in total protein was observed in both heavy as well as moderate drinkers with ALD when compared with normal healthy control. Heavy drinkers were found significantly low value compared to moderate drinkers. Ethanol consumption hinders the rate of hepatic protein catabolism (slows down). Similar results also reported by Das SK and Vasudevan DM, [38]. Large amount of alcohol for a long period may develop abnormal liver functions, however, in the present study no major significant difference was observed in parameters related to liver function on comparison between heavy and moderate drinkers except serum total bilirubin.

This study found significantly increased levels of total cholesterol, triglyceride, LDL-C and VLDL-C on comparison between ALD with moderate drinkers and healthy controls, except HDL-C. Similar finding was also noted in ALD with heavy drinkers when it compared to healthy controls. On comparison of moderate drinkers with heavy drinkers for lipid profile parameters, the significant difference was found only for LDL cholesterol. However, a long-term intake of alcohol results in perceptive derangements of lipid metabolism [39]. The present study also showed significantly increased level of plasma MDA (p<0.001) in both ALD with heavy and moderate drinkers in comparison to healthy controls. Similar result also documented by Gupta S et al., in there study [40]. The present study observed a significant difference in MDA levels between moderate and heavy drinkers. As MDA is a known biomarker of oxidative stress and several animal and human studies strongly favuor the possibility of oxidative stress in the pathogenesis of ALD due to the excessive production of alcohol-mediate ROS [41,42]. The present study also showed significant decreased antioxidants such as SOD and CAT in both ALD with heavy and moderate drinkers in comparison to healthy controls. However, on comparison of antioxidant parameter between moderate and heavy drinkers, no significant result observed. Previous studies have also reported significantly decreased activity of SOD and CAT in ALD patients as compared to the controls [43-47].

According to the previous studies there are a relationship between the incidence of alcoholic liver cirrhosis and alcohol intake. Therefore, these studies suggested that 60 g of alcohol intake per day in men and 20 g of alcohol intake per day in women prompts the risk of liver cirrhosis. Furthermore, daily alcohol intake is more injurious as compared to binge drinking [36,48]. For a log period alcohol consumption might be responsible for development of ALD despite this, in our study no significant changes were noted in biochemical, antioxidant and oxidative parameters between heavy and moderate drinkers.

Limitation(s)

The study did not address the possible confounding effect of smoking, gender, and morphological patterns of liver cirrhosis. The type of beverage is also an important factor which may have influenced the result.

CONCLUSION(S)

This study showed increased production of MDA and reduced scavenging ability of SOD and CAT in ALD patients with moderate as well as heavy drinkers. It may be a consequence of alcohol-

mediated increased production of free radicals. As alcohol consumption is directly associated with biochemical as well as oxidant-antioxidant alteration in liver cells therefore, monitoring of biochemical parameters, MDA, SOD and CAT might be more helpful in detecting the severity of alcoholic liver injury.

REFERENCES

[1] Lieber CS. Alcoholic liver disease: New insights in pathogenesis lead to new treatments. J Hepatol. 2000;32(1):113-28.
[2] Osna NA, Donohue TM Jr, Kharbanda KK. Alcoholic liver disease: Pathogenesis and current management. Alcohol Res. 2017;38(2):147-61.
[3] Singh RB, Ghosh S, Niaz MA, Rastogi V, Wander GS. Validation of tobacco and alcohol intake for the five city study and a proposed classification for Indians. J Assoc Physicians India. 1998;46:587-91.
[4] Patil AM, Mohammed AK, Saeed M, Sajanar BB. Study of alcoholic liver cirrhosis in hospital based patients, Bijapur, Northern Karnataka, India. IJCMAAS. 2015;7(1):16-20.
[5] Singh M, Gupta S, Singhal U, Pandey R, Aggarwal SK. Evaluation of the oxidative stress in chronic alcoholics. J Clin Diagno Res. 2013;7(8):1568-71.
[6] Crabb DW. Pathogenesis of alcoholic liver disease: Newer mechanisms of injury. Keio J Med. 1999;48:184-88.
[7] Crawford JM. Histologic findings in alcoholic liver disease. Clin Liver Dis. 2012;16:699-716.
[8] Theise ND. Histopathology of Alcoholic liver disease. Clin Liver Dis. 2013;2:64-67.
[9] Prasad PT, Apte IC, Bhute AK. Biochemical evaluation of patients of alcoholic liver disease and non-alcoholic liver disease. Ind J Clin Bioche. 2014;29(1):79-83.
[10] Nalpas B, Hispard E, Thepot V, Pot S, Dally S, Berthelot P. A comparative study between carbohydrates-deficient transferring and gamma-glutamyl transferase for the diagnosis of excessive drinking in a liver unit. J Hepatol. 1997;27:127-35.
[11] Lonardo A, Romagnoli D. Gamma glutamyl transferase: A novel cardiovascular outfit for an old liver test. Indian J Med Res. 2016;143(1):04-07.
[12] Shaffer EA, Thomson ABR. Cirrhosis of liver. First principles of gastroenterology: The basis of disease and an approach to management. 3rd ed. Edmonton: J Canadian Assoc Gastroentero Heaptol. 1997;520-24.
[13] Gowda S, Desai PB, Hull VV, Math AK, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. Pan Afr Med J. 2009;3(17):01-18.
[14] Kundu D, Roy A, Mandal T, Bandyopadhyay U, Ghosh E, Ray D. Oxidative stress in alcoholic and viral hepatitis. N Am J Med Sci. 2012;4(9):412-15.
[15] Dowman JK, Tomlinson JW, Newsome PN. Systematic review: The diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2011;33(5):525-40.
[16] Daeppen JB, Schoenfeld-Smith K, Smith TL. Characteristics of alcohol dependent subjects with very elevated levels of Gamma-Glutamyltransferase (GGT). J Stud Alcohol. 1999;60(5):589-94.
[17] Majhi S, Baral N, Lamsal M, Mehta KD. De Ritis ratio as diagnostic marker of alcoholic liver disease. Nepal Med Coll J. 2006;8(1):40-42.
[18] Eckardt JM, Harford F, Kaelber TC. Health hazards associated with alcohol consumption. J Am Med Assoc. 1981;246(6):648-66.
[19] Ray AN, Barman DC, Pandit N. Clinical study on haematological profile abnormalities in alcoholic liver disease patients in a tertiary teaching hospital in North East India. J Evid Based Med Healthc. 2019;6(36):2455-60.
[20] Vaswani M, Hemraj P, Desai NG, Tripathi BM. Lipoid profile in alcoholic dependence. Indian J Psychiat. 1997;39 (l):24-28.
[21] Vasudevan DM, Sreekumari S. Iso-enzymes and clinical enzymology. Textbook of Biochemistry (for medical students). 4th edition, New Delhi: Jaypee Brothers Medical Publishers (P) Ltd, 2005; 57.
[22] Zakhari S. Overview: How is alcohol metabolized by the body? Alcohol Res Health. 2006;29(4):245-54.
[23] Worrall S, Thiele GM. Protein modification in ethanol toxicity. Adverse Drug React Toxicol Rev. 2001;20(3):133-59.
[24] Wu D, Cederbaum AI. Alcohol, oxidative stress, and free radical damage. Alcohol Res Health. 2003;27(4):277-84.
[25] Sies H. Oxidative stress: Oxidants and antioxidant. Exp physiol. 1997;82(2):291-95.
[26] Kessova IG, Ho YS, Thung S, Cederbaum AI. Alcohol induced liver injury in mice lacking Cu, Zn-Superoxide dismutase. Hepatol. 2002;38(5):1136-45.
[27] Bruha R, Dvorak K, Petrtyl J. Alcoholic liver disease. World J Hepatol. 2012;4(3):81-90.
[28] Makwane H, Paneri S, Varma M, Jha RK. Study of biochemical changes, antioxidant status and oxidative stress in patients with alcoholic liver disease. Int J Intg Med Sci. 2018;5(4):603-06.
[29] Usharani B, Vennila R, Nalini N. Biochemical changes in Alcoholics- A case control study. IJRPBS. 2012;3(1):201-05.
[30] Paton A Asking the right questions. In: ABC of Alcohol, Ed. A. Paton, BMJ Publishing Group, Tavistock square, London; 1994: p.14.
[31] Jean CD, Maryse T, Marie JF. Plasma malondialdehyde levels during myocardial infarction. Clin Chem Acta. 1983;129:319-22.
[32] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;469-74.
[33] Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-26.
[34] Addolorato G, Capristo E, Greco AV, Stefanini GF, Gasbarrini G. Influence of chronic alcohol abuse on body weight and energy metabolism: Is excess ethanol consumption a risk factor for obesity or malnutrition? J Intern Med. 1998;244(5):387-95.
[35] World MJ, Ryle PR, Jones D, Shaw GK, Thomas AD. Different effects of alcohol intake and poor nutrition on body weight and fat stores. Alcohol Alcohol. 1984;19(4):281-90.
[36] Vozzo CF, Welch DN, Marrero CR, Fairbanks KD. Alcoholic liver disease. Published: June 2018.
[37] Alatalo P, Koivisto H, Puukka K, Hietala J, Anttila P, Bloigu R, et al. Biomarkers of liver status in heavy drinkers, moderate drinkers and abstainers. Alcohol Alcohol. 2009;44(2):199-203.
[38] Das SK, Vasudevan DM. Biochemical diagnosis of alcoholism. Indian J Clin Biochem. 2005;20(1):35-42.
[39] Sabesin SM. Lipid and lipoprotein abnormalities in alcoholic liver disease. Circulation. 1981;64:72-84.
[40] Gupta S, Pandey R, Katyal R, Aggrwal HK, Aggrwal SK. Lipid peroxide and antioxidant status in alcoholic liver disease. Indian J Clin Biochem. 2005;20(1):67-71.
[41] Par A, Roth E, Rumi G, Kovacs Z, Nemes J, Mozsik G. Oxidative stress and antioxidant defenses in alcoholic liver disease and chronic hepatitis C. Orv Hetil. 2000;141(30):1655-59.
[42] Nalini G, Hariprasad C, Narayanan VA. Oxidative stress in alcoholic liver disease. Indian J Med Res. 1999;110:200-03.
[43] Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidant and human disease; where are we now? J Lab Clin Med. 1992;119(6):598-620.
[44] Makwane H, Varma M, Jha RK. Study of oxidative stress and hsCRP in alcoholic and non-alcoholic fatty liver disorders. Sch. J. App. Med. Sci. 2018;6(2):563-69.
[45] Chari S, Gupta M. Status of blood antioxidant enzymes in alcoholic cirrhosis. Indian J Physio Pharma. 2003;47(3):343-46.
[46] Janani AV, Suprapaneni KM. Antioxidant vitamins and enzyme status in patients with alcoholic liver disease. J Clin Diab Res. 2010;(4):2742-47.
[47] Ledig M, Doffoel M, Doffoel S, Kopp P, Bockel R, Mandel P. Blood cell superoxide dismutase and enolase activities as markers of alcoholic and nonalcoholic liver diseases. Alcohol. 1988;15(5):387-91.
[48] Grant BF, Dufour MC, Harford TC. Epidemiology of alcoholic liver disease. Semin Liver Dis. 1988;8(1):12-25.

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