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Original Research Article

Oxidative Stress in Alcoholic Liver Disease

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ABSTRACT

Background: Alcoholic Liver disease is mainly of three types:- Alcoholic fatly liver; Alcoholic hepatitis; and alcoholic cirrhosis. During the metabolism of alcohol in the liver, produces highly reactive molecules that can destroy vital cell components through a chemical process called oxidation. Oxidative stress increases the rate of production of free radicals hence induces lipid peroxidation.
Antioxidants are natural defence mechanism. Existing in our system and these are capable of scavenging the deleterious free radicals.
Aims: To study the activity of serum GGT Serum, MDA and GSH in whole blood in the patients with alcoholic liver disease and its comparison with controls and to study the correlation of serum MDA with serum GGT and blood GSH in the patients with alcoholic liver disease.
Material And Method: The study was conducted on 50 diagnosed cases of alcoholic liver disease visiting Rajindra Hospital, Patiala and 50 healthy subjects of matched age & sex served as controls and Biochemical Investigations of Serum GGT, Serum MDA and GSH in whole blood were conducted in the department of Biochemistry Govt. Medical College Patiala and the results were statistically analysed.
Results: The mean values of serum GGT in study group and control group were 20.80±6.38 and 234.44±204.31(IU/L) respectively (Normal value of GGT 0-50 (IU/L), mean values of serum MDA in study group and control group were 22.00±5.03 and 53.90±11.43(µmol/L) respectively and mean values of blood glutathione in control group and study group were 41.6±4.51 and 18.40±2.39(mg%) respectively the decrease mean blood Glutathione level in study group as compared to mean blood Glutathione level in control group shows statistically significant association (p value < 0.001).
Conclusion: Our study shows that there was increased levels of Serum MDA, Serum GGT and decreased levels of blood glutathione in the study group and there was a positive correlation between Serum MDA levels and Serum GGT levels in the study group and there was a negative correlation between serum MDA levels blood glutathione levels in the study group. This correlation predicts that during increased oxidative stress, the GSH was utilized to counter the oxidative stress due to alcoholic liver disease.

Keywords: GGT (gamma glutamyl transferase) ALD (Alcoholic liver disease), GSH (reduced glutathione), LPO (Lipid Peroxidation) MDA (Malonyldialdehyde), ROS(Reactive oxygen species), SOD (superoxide dismutase).

INTRODUCTION

Alcoholic liver disease is the most prevalent form of liver disease in the western world. The risk increases rapidly when ethanol consumption exceeds 80 g/day for more than one year (Eighth special report to the US congress on Alcohol and Health U.S. Deptt of Health and Human Services, 1993). A co-existent chronic liver disease (e.g. Hepatitis B or C) also may increase susceptibility to and the severity of alcoholic liver disease (Sherlock 1995). [1]
Alcoholic liver disease is mainly of three types: alcoholic fatty liver; alcoholic hepatitis; and alcoholic cirrhosis (Ishak et al 1991). [2]

Poli et al (1987) [3] suggested the role of free radicals in liver injury. Free radicals cause peroxidative breakdown of membrane lipids that can alter mitochondrial functions through oxidation of pyridine nucleotides and consequent alteration in calcium uptake. Several enzymatic functions of the endoplasmic reticulum are also affected, thereby leading to liver cell injury and eventually fibrositis.

Lipid peroxidation is a process of hydrolysis, which produces aldehyde, the most represented being malonyldialdehyde which reacts with thiobarbituric acid and whose concentration is considered a marker of lipid peroxidation (Bianchi et al 1997). [4]

Free radicals attack unsaturated fatty acids presents in cell membrane leading to formation of lipid peroxides. The products of lipoxidation may act as initiators or promoters of cell injury. These products include malonyldialdehyde (MDA) and oxygen free radicals that are capable of causing widespread tissue damage (Ames 1983). [5]

MDA is a marker of free radical mediated oxidative stress: It is a decomposition product of the oxidized polyunsaturated fatty acids. This three carbon dialdehyde has been proposed to arise from fatty acid hydroperoxides via several mechanisms. The most frequent precursors of MDA are the five-membered hydroperoxy epidioxides (Endoperoxides) and 1,3 – dihydroperoxides (Pyror et al 1976). [6] These lipid products are unstable and undergo decomposition to secondary lipid peroxidation products, such as malonyldialdehyde (Chiu et al 1982). [7]

The detection of MDA using its reactions with thiobarbituric acid (TBA) (has been most widely used as an indicator of LPO. The Lipid peroxidation plays an important mechanism in the pathogenesis of alcoholic liver disease (Shaw et al 1981). [8]

GGT: Basically it is a glycoprotein in nature (Meister and Anderson 1983). [9] GGT catalyses the transfer of gamma glutamyl group from gamma glutamyl peptides to another peptide or L-amino acids or for removal of glutamyl group from compounds containing it. It changes glutathione into glutamic acid and cysteinyl glycine (Meister and Tate 1976). [10]

Glutathione+amino acid $\xrightarrow{\text{GGT}}$ Glutamic acid+cysteinyl glycine

A significant increased level of gamma glutamyl transferase (GGT) was observed in alcoholic liver disease (Nalini et al 1999). [11]

ANTIOXIDANT SYSTEM: There are three main groups of antioxidants: Primary antioxidants, Secondary antioxidants and tertiary antioxidants. Primary antioxidants prevent the formation of new radical species, either by converting existing free radicals into harmless molecules or by preventing formation of fresh free radicals from other molecules e.g. superoxide dismutase (SOD) converts superoxide (O_2) to hydrogen peroxide (H_2O_2),glutathione peroxidase converts H_2O_2 to less harmful molecules, metal binding proteins like ferritin and ceruloplasmin limit the availability of Fe^{++}ions necessary for the formation of hydroxyl ($OH^{\cdot}$) radicals (Sainani et al 1997). [12]

GLUTATHIONE: Glutathione (GSH) plays an important role in free radical scavenging strategies of the body (Arias and Jakoby 1975). [13] Glutathione is an important antioxidant, as it can destroy reactive oxygen species and other free radicals by various enzymatic as well as non-enzymatic processes (Cadenas 1989). [14]

A significant inverse relationship between liver lipoperoxidation and the levels of GSH in alcoholics exist (Videla et al 1980). [15] A significant decreased level of reduced GSH was observed in alcoholic liver disease (Nalini et al 1999). [11]

MATERIAL AND METHODS

The present study (analysis) was conducted on 50 diagnosed cases of alcoholic liver disease visiting Rajindra Hospital, Patiala and 50 healthy subjects of

matched age & sex served as controls. The study group was further subdivided into three sub groups on the basis of stage of alcoholic liver disease. Out of them, 25 cases (50%) (sub group IIA) were suffering from alcoholic fatty liver, 20 cases (40%) (sub group IIB) were suffering from alcoholic hepatitis and 5 cases (10%) (sub group IIC) were having alcoholic liver cirrhosis.

Diagnostic criteria for a case of alcoholic liver disease is as under:

- History of significant alcohol intake]
- Clinical assessment/examination
- Liver function test
- Ultrasound /endoscopy wherever it is necessary.

Patients suffering from disease like diabetes mellitus, rheumatoid arthritis, ischemic heart disease and stroke which are known to cause increased levels of malonyldialdehyde were excluded from the present study.

Routine and special investigations were done in study group as well as in control group.

Special Investigations

In all the cases and the controls of this study, the levels of following special investigations were carried out:

i. Serum Gamma-glutamyl transferase (GGT)
ii. Serum Lipid peroxidation (MDA)
iii. Glutathione in whole blood(GSH).

Sample Collection

All blood samples were collected with a dry, sterilized syringe and needle from median cubital vein under aseptic conditions. A total of 10 ml of blood was collected, 5ml in a citrated vial for estimating blood glutathione levels and 5ml in a plain vital for estimating serum GGT and serum MDA levels.

STATISTICAL ANALYSIS AND RESULTS

The present study was conducted to evaluate free radical stress (oxidative stress) and antioxidant system in alcoholic liver disease.

To compare the values of serum GGT, serum MDA and blood GSH levels with study group, fifty age and sex matched healthy controls were taken. This statistical analysis and results of the study group and control group are as under:

TABLE 1 SHOWS THE COMPARISON OF SERUM GGT LEVELS IN CONTROL AND STUDY GROUPS

S.NO	GROUP	NO	RANGE(IU/L)	MEAN±SD(IU/L)
I	Control	50	14-50	20.80±6.38
II	Study:			
IIA	Alcoholic fatty liver	25	55-180	124.96±31.91
IIB	Alcoholic hepatitis	20	200-280	237.90±24.90
IIC	Alcoholic cirrhosis	5	380-970	768.00±237.87
III	Total study group	50	55-970	234.44±204.31

Statistical analysis

Comparison	't' value	'p'value	Significance
I & IIA	22.34	<0.001	Highly significant
I & IIB	57.63	<0.001	Highly significant
I & IIC	24.27	<0.001	Highly significant
I & III	7.50	<0.001	Highly significant

The above table shows that the mean GGT levels in the control group were 20.80±6.38 IU/L and in the total study group, it was 234.44±204.31 IU/L. In the study sub groups IIA, IIB, and IIC. The mean serum GGT levels were 124.96±31.91IU/L, 237.90±24.90 IU/L and 768.00±237.87 IU/L respectively.

On statistical analysis, the mean GGT levels in the total study group were significantly higher ($p<0.001$) as compared to the mean GGT levels of the control group.

On further statistical analysis, the mean GGT levels in the study sub groups (IIA, IIB, IIC) were significantly higher

(p<0.001) as compared to the mean GGT levels in the control group.

TABLE 2 SHOWS THE COMPARISON OF SERUM MDA LEVELS IN CONTROL AND STUDY GROUPS

S.NO	GROUP	NO	RANGE(µmol/L)	MEAN±SD
I	Control	50	14-40	22.00±5.03
II	Study:			
IIA	Alcoholic fatty liver	25	40-61	51.80±5.86
IIB	Alcoholic hepatitis	20	50-80	61.60±7.35
IIC	Alcoholic cirrhosis	5	75-88	83.60±5.02
III	Total study group	50	40-88	53.90±11.43

Statistical analysis

Comparison	't' value	'p'value	Significance
I & IIA	22.85	<0.001	Highly significant
I & IIB	25.90	<0.001	Highly significant
I & IIC	26.08	<0.001	Highly significant
I & III	20.87	<0.001	Highly significant

The above table shows that the mean serum MDA levels in the control group were 22.0±5.03 µmol/L and in the total study group, it was 53.90±11.43 µmol/L. In the study sub groups IIA, IIB and IIC. The mean serum MDA levels were 51.80±5.86 µmol/L, 61.60±7.35 µmol/L and 83.60±5.02 µmol/L respectively.

On statistical analysis, the mean serum MDA levels in the total study group were significantly higher (p<0.001) as compared to the mean serum MDA levels of the control group.

On further statistical analysis, the mean serum MDA levels in the study sub groups (IIA, IIB, IIC) were significantly higher (p<0.001) as compared to the mean serum MDA levels in the control group.

TABLE 3 SHOWS THE COMPARISON OF BLOOD GLUTATHIONE LEVELS IN CONTROL AND STUDY GROUPS

S.NO	GROUP	NO	RANGE(mg%)	MEAN±SD(mg%)
I	Control	50	30-50	41.06±4.51
II	Study:			
IIA	Alcoholic fatty liver	25	18-24	19.92±1.55
IIB	Alcoholic hepatitis	20	14-21	17.55±1.70
IIC	Alcoholic cirrhosis	5	13-16	14.20±1.30
III	Total study group	50	13-24	18.40±2.39

Statistical analysis

Comparison	't' value	'p'value	Significance
I & IIA	22.68	<0.001	Highly significant
I & IIB	22.57	<0.001	Highly significant
I & IIC	13.14	<0.001	Highly significant
I & III	31.36	<0.001	Highly significant

The above table shows that the mean levels of blood glutathione in the control group were 41.06±4.51 mg% and in the total study group, it was 18.40±2.39 mg%. In the total study sub groups IIA, IIB and IIC. The mean blood glutathione levels were 19.92±1.55 mg%, 17.55±70 mg% and 14.20±1.30 mg% respectively.

On statistical analysis, the mean blood glutathione levels in the total study group were significantly lower (p<0.001) as compared to the mean blood glutathione levels of the control group.

On further statistical analysis, the mean blood glutathione levels of the study sub groups (IIA, IIB, IIC) were significantly lower (p<0.001) as compared to the mean blood glutathione levels in the control group.

TABLE 4 SHOWS THE COEFFICIENT OF CORRELATION BETWEEN SERUM MALONYLDIALDEHYDE LEVELS (µmol/L) AND SERUM GGT LEVELS (IU/L) IN STUDY GROUPS

PARAMETERS	MEAN±SD	CO-EFFICIENT OF CORRELATION	'P' VALUE	SIG.
MDA (n=50)	53.90±11.43	+0.81	<0.01	HS
GGT (n=50)	234.44±204.31			

Line of regression:

Y=14.37x-612.71

From this table, it is evident that the coefficient of correlation between serum MDA levels and serum GGT levels is +0.81. This indicates that there is a positive correlation between serum MDA levels and serum GGT level and statistically it is highly significant (p<0.01).

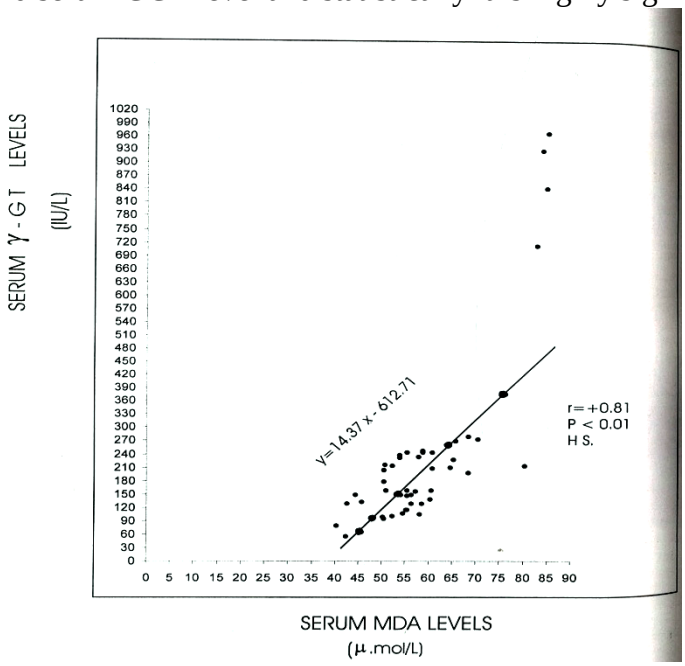

Figure 1. Shows the scatter diagram showing co-relation between Serum MDA and Serum GGT Levels.

TABLE 5 SHOWS THE COEFFICIENT OF CORRELATION BETWEEN SERUM MALONYLDIALDEHYDE LEVELS (µmol/L) AND BLOOD GLUTATHIONE LEVELS (mg%) IN STUDY GROUPS

PARAMETERS	MEAN±SD	CO-EFFICIENT OF CORRELATION	'P' VALUE	SIG.
MDA (n=50)	53.90±11.43	-0.77	p<0.01	HS
GGT (n=50)	18.40±2.39			

Line of regression:

Y = -0.16+27.88

From this table, it is evident that the coefficient of correlation between serum MDA levels and blood glutathione levels is -0.77. This indicates that there is a negative correlation between serum MDA levels and blood glutathione level and statistically it is highly significant (p<0.01).

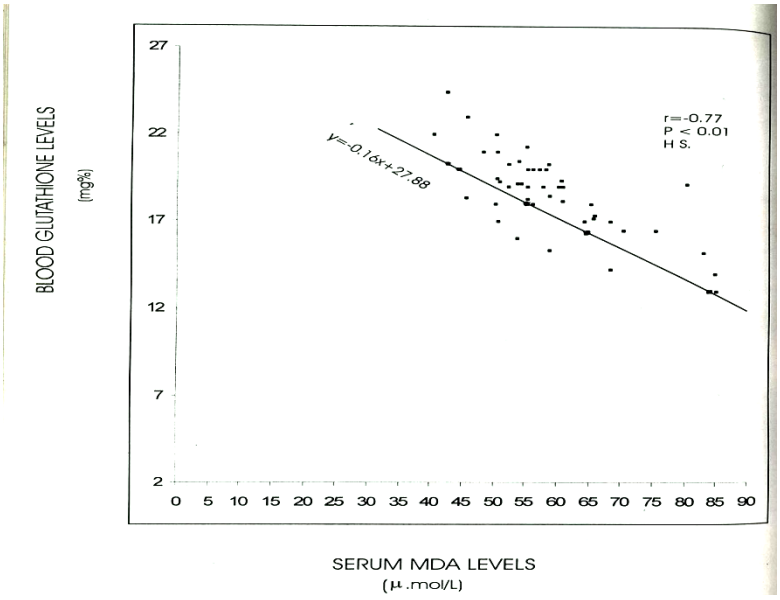

Figure 2. Shows the scatter diagram showing co-relation between Serum MDA and Blood Glutathione Levels.

DISCUSSION

The present study was undertaken with an aim to evaluate the status of free radical oxidants (MDA) and antioxidants (GSH) in the fifty diagnosed cases of alcoholic liver disease. The activity of GGT glutamyl transferase was also evaluated in alcoholic liver disease.

Out of the 50 cases of study group, 25(50%) cases were of alcoholic fatty liver, 20 (40%) cases were of alcoholic hepatitis and 5 (10%) were of alcoholic cirrhosis.

Lipid Peroxidation : Halliwell and Gutteridge (1989) [16] started that oxidative stress means peroxidant injury overwhelming antioxidant defence mechanism both enzymatic and non-enzymatic oxidative stress leads to peroxidation of lipids and denaturation of proteins.

Cheeseman and Slater (1993) [17] concluded that measurement of malonyldialdehyde (MDA) and diene conjugates in serum and various tissues of the patients can act as a guide for the accurate assessment of degree of lipid peroxidation.

Nalini et al (1999) [11] determined the mean levels of serum MDA in 22 cases of alcoholic liver disease and the mean serum MDA levels in the study group were 3.82±1.65 nmol/ml as compared to the mean serum MDA levels in the control was 2.49±1.42 nmol/ml.

In the present study, the mean serum MDA levels in study group 53.90±11.43 $\mu mol/L$ as compared to the mean serum MDA levels were 22.00±5.03 $\mu mol/L$ in the control group ($p<0.001$). Thus the findings in the present study regarding the serum MDA levels in alcoholic liver disease are consistent with the previous study conducted by Nalini et al (1999) [11] in the same category of patients.

Blood Glutathione: Glutathione is a tripeptide: plays an important role in free radical scavenging strategies of the body (Arias and Jakoby 1975). [13] Cadenas (1989) [14] studied that glutathione is an important antioxidant as it can destroy reactive oxygen species and other free radicals by various enzymatic as well as non- enzymatic processes.

Comporti (1985) [18] studied that glutathione plays an important role as a co-factor for enzymes invoved in protecting membranes against oxidative dagmage, maintaining membrane proteins and ascorbic acid in reduced form.

Nalini et al (1999) [11] determined the GSH levels in the haemolysate in 22 cases of alcoholic liver disease and the mean levels in the study group were 2.03±0.99 µ g/mg Hb and in the control group it was 3.66±0.9 µg/mg Hb.

In the present study the mean blood glutathione levels in the study group were 18.40±2.39 mg% and in the control group it was 41.06±4.51 mg% ($p<0.001$).Thus the findings in the present study regarding the levels of the blood glutathione in alcoholic liver disease are consistent with the previous study conducted by Girlamo et al (1966) [19] and Nalini et al (1999) [11] in the same category of patients.

Szczeklik and Orlowski (1961) [20] reported the increased activity of GGT in cases of alcoholic liver Disease. Rosalki (1972) [21] reported the increased activity of GGT in 71.9% of cases of alcoholic liver disease. Lum ann Gambino(1972), [22] Boone and Tietz (1977) [23] repoted the increased activity of GGT in 80% of cases of alcoholic liver disease.

Nemesanszky and john (1985) [24] reported the increased activity of GGT in 96% of cases of alcoholic liver diseases.

Nalini et al (1999) [11] determined the activity of GGT in 22 cases of alcoholic liver disease. The man levels were 97.80±90.21 IU/L in the study group as compared to the mean levels 31.00±28.60 IU/L in the control group.

In the present study the mean GGT levels int the study group were 234.44±201.31 IU/L as compared to the mean levels 20.80±6.38IU/L in the control group ($p<0.001$).thus the findings in the present study regarding the serum levels GGT in alcoholic liver disease are consistent with the previous study

conducted by Nalini et al (1999) [11] in the same category of patients.

Correlation between serum malonyldialdehyde and blood glutathione levels

Radicals formed as a result of oxidative stress in alcoholic liver disease, trigger a series of reactions leading to generation of many different radical species. Antioxidants neutralize these free radicals so that they cannot react with additional substrate thereby, getting consumed during free radical stress. So, there should be a negative correlation between serum malonyldialdehyde levels and blood glutathione levels.

In the present study, on analyzing study group, it was observed that the value of coefficient f correlation between serum MDA levels and blood glutathione levels was -0.77. This showed a negative correlation between the two which was found to be highly significant ($p<0.01$).

Correlation between serum malonyldialdehyde and serum GGT Levels.

Free radicals play an important role in the pathogenesis of alcoholic liver disease. Ethanol causes liver injury there is increase in the activity of GGT. So, during oxidative stress in alcoholic liver disease, there is increased level of MDA and GGT. So, there should be positive correlation between serum malonyldialdehyde levels.

In the present study, on analyzing study group, it was observed that the value of coefficient of correlation between serum MDA levels and serum GGT levels was +0.81. This showed a positive correlation between the two which was found to be highly significant ($p<0.01$).

CONCLUSIONS

Our study shows that:-

The mean value of serum MDA levels in the study group was elevated which was statistically highly significant as compared to the control group (53.90±11.43 $\mu mol/L$ Vs 22.00±5.03 $\mu mol/L$, $p<0.001$). The mean blood glutathione (GSH) levels in the study group significantly lower as compared to the control group(18.40±2.39 mg% Vs 41.06±4.51 mg%, $p<0.001$.The mean serum GGT Glutamyl transferase levels in the study group were significantly elevated as compared to the control group (234.44±204.31 IU/L Vs. 20.86±6.38 IU/L; $p<0.001$). There was a positive correlation between serum, MDA levels and serum GGT levels in the study group ($r=+0.81$) and it was found to be highly significant ($p<0.01$).There was a negative correlation between serum MDA levels and blood glutathione levels in the study group ($r=-0.77$) and it was found to be highly significant ($p<0.01$).

Hence, the present study demonstrates statistically significant elevation of serum MDA, serum GGT and reduction of blood glutathione levels in all cases of alcoholic liver disease. The elevation of serum MDA, serum GGT and reduction of blood glutathione levels were more pronounced in cases of study sub group IIC (alcoholic cirrhosis). This suggests the involvement of free radical injury in the pathogenesis of alcoholic liver disease.

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