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ALCOHOLIC LIVER DISEASE (ALD);

IMPLICATION OF OXIDATIVE STRESS & EXTRAPOLATIVE FACTORS IN PATIENTS SUFFERING, UPDATE FROM LOCAL POPULATION OF PUNJAB-PAKISTAN

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ABSTRACT… Objective: The aim of the present study was to investigate the changes in biochemical parameters including lipid profile, liver and kidney profile as well as oxidative stress profile, particularly in patients suffering from alcoholic liver disease (ALD). **Study design:** Fifty chronic alcoholics admitted for treatment to the in-patient wards at Jinnah Hospital, Lahore-Pakistan. Chronic alcoholics with alcohol abuse for more than four to five years, and with or without clinical complications, were included. Apparently twenty healthy individuals served as control. **Period:** 2012-2013. **Materials and methods:** Various circulating biochemical biomarkers including renal profile, hepatic and lipid profile were evaluated. Moreover, stress markers (MDA. SOD, GSH and catalase) were also investigated. **Results:** A very strong direct and indirect correlation of ALP was found with TB, MDA and GSH (r=.950**, r=.929** and r=-.967** respectively, P<0.01). MDA was observed having very strong indirect correlation with GSH and catalase (r=-.909** and r=-.777** respectively, P<0.01). **Conclusion:** All parameters in combinations may be useful indicator or may be good and reliable biochemical markers for identification and determination of severity of alcoholic liver diseases (ALD). The damaging of hepatocytes due to the consumption of alcohol disturbs almost all types of biochemical coordination in the biological system.

Key words: Alcoholic Liver Disease (ALD), Total Bilirubin, lipid peroxidation, MDA, ALT, AST, ALP

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INTRODUCTION

The liver is one of the largest organs in the body, weighting 3.3 pounds. It has a distinctive property that liver cells can be regenerated themselves. Because of this, damage to the liver cells cannot appear until or unless the damage caused is considerable and extensive.[1] It's a very important organ in the body as its functions are very essential to life. Liver primarily filters circulating blood and thus removing and destroying toxic substances. The major function of liver is to secrete bile in small intestine which helps in digestion and absorption of fats. The protein products manufactured as a result of metabolism are also converted by liver into urea so that they can be excreted via kidney. Liver also regulates blood clotting mechanisms. Because of the regenerative ability of liver this important organ can survive harsh condition during life time.[2]

Hepatitis C is much considered as main cause for liver cirrhosis. Fat deposition in liver can be occurred in almost all heavy drinkers. This fatty liver can also be seen in nonalcoholic that drinks casually. Fatty liver is seemed to be reversible and this cannot leads towards serious disease.[1] Liver transplantation is one of the effective therapies available to some patient.[3] Liver cirrhosis is indolent disease, most of the patient show asymptomatic disease till the onset of last stage.[4] Liver cirrhosis is pathological disease that is characterized by irreversible chronic injury of hepatic cells in association of vast fibrosis.[5]

Objective

The aim of the present study was to investigate the changes in biochemical parameters in patients with alcoholic liver disease (ALD).

MATERIALS AND METHODS

	Groups	(n)
A	Non-Alcoholics (Served as control)	20
B	Alcoholics (01-05 Yrs Exposure time)	4
C	Alcoholics (06-10 Yrs Exposure time)	41
D	Alcoholics (11 Yrs and above Exposure time)	5

Table-I. Study Design

SOURCE OF DATA

Fifty chronic alcoholics admitted for treatment to the in-patient wards at Jinnah Hospital, Lahore-Pakistan. Detailed history of alcohol intake was collected including clinical complications. Apparently twenty healthy individuals served as control. Chronic alcoholics with alcohol abuse for more than four to five years, and with or without clinical complications, were included. Alcoholics with smoking, occasional drinkers and with any systemic illness were excluded from the control group.

BIOCHEMICAL ASSAYS

The estimation of AST, ALT and ALP were estimated by following principle by using commercially available Bio Meraux and Randox kits. Hemoglobin concentration was determined using cyanmeth reagent.[6] Urea in serum was estimated by the kinetic method.[7] Creatinine level was estimated by rate of change in absorbance using alkaline picrate.[8] Total Bilirubin levels of serum were measured by the method of Jendrassik and Groff.[9]

Liver GSH was estimated according to the method of Ellman.[10] Catalase was assayed according to the method of Aebi.[11] Lipid peroxidation in liver tissues was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa.[12] Superoxide dismutase (SOD) activity was determined by the method of Kakkar.[13]

STATISTICAL ANALYSIS

ANOVA was applied to check statistically significant ($P<0.05$) difference among the groups. Data was represented by mean±SD.

RESULTS

Hematological Profile of Alcoholics vs. Non Alcoholics

The results regarding hemoglobin and RBC profile in Table-II demonstrating statistically highly significant differences and consistent decreasing (hemoglobin) and increasing (RBC) pattern between and within the alcoholics and non-alcoholics subjects (P=.05, .01and .001 respectively). The lowest value (10.30%) of hemoglobin was recorded in alcoholics in group D (11 Yrs and above Exposure time) and highest (14.90%) in group A (served as control). A slightly decreasing trend was observed in other groups B-C (11.30%, 10.78%) respectively. The highest value (5.97) of RBC count (x10) was found in non-alcoholics. Increasing trend of RBC count (x10) was observed from groups B-D (3.75, 4.02 and 4.26) respectively but this increasing trend presenting lower values as compared to group A (served as control).

PARA-METERS	GROUPS	MEAN±SD	(n)	P-VALUE
Hb	A	14.90±0.99	20	.000*
	B	11.30±1.39	4	
	C	10.78±1.20	41	
	D	10.30±1.69	5	
RBC	A	5.97±0.49	20	.000*
	B	3.75±0.57	4	
	C	4.02±0.43	41	
	D	4.26±0.04	5	

Table-II. Hematological Profiles of Alcoholics vs. Non Alcoholics

Blood Urea and Creatinine Profile of Alcoholics vs. Non Alcoholics

The data regarding blood urea and creatinine profile in Table-III reflecting highly significant differences but inconsistent increasing and decreasing pattern between and within the alcoholics and non-alcoholics subjects (P=.05, .01and .001 respectively). The lowest value (0.93 mg/dl) of

blood creatinine (Normal range, 0.8-1.7 in Males) was recorded in non-alcoholics and highest (1.96 mg/dl) in group B (01-04 Yrs Exposure time). A slightly decreasing trend was observed in other groups (C and D). Increasing trend of blood urea was noted from groups A-C (20.73, 22.49 and 24.37 mg/dl) respectively with a slightly decreasing value (23.94 mg/dl) respectively.

PARA-METERS	GROUPS	MEAN±SD	(n)	P-VALUE
Urea	A	20.73±1.48	20	.000*
	B	22.49±2.20	4	
	C	24.37±2.12	41	
	D	23.94±2.57	5	
Creatinine	A	0.96±0.19	20	.000*
	B	1.96±0.06	4	
	C	1.91±0.25	41	
	D	1.94±0.45	5	

Table-III. Renal Profiles of Alcoholics Vs Non Alcoholics

Hepatic Profile of Alcoholics vs. Non Alcoholics

PARA-METERS	GROUPS	MEAN±SD	(n)	P VALUE
ALT	A	24.00±5.69	20	.000*
	B	36.75±19.73	4	
	C	52.04±21.16	41	
	D	62.60±33.90	5	
AST	A	20.25±5.21	20	.000*
	B	48.50±9.32	4	
	C	79.17±31.09	41	
	D	138.40±25.89	5	
ALP	A	207.83±6.28	20	.000*
	B	369.16±13.53	4	
	C	389.25±23.20	41	
	D	393.07±36.83	5	
TB	A	0.59±0.07	20	.000*
	B	2.00±0.02	4	
	C	2.05±0.20	41	
	D	2.09±0.15	5	

Table-IV. Hepatic Profiles of Alcoholics vs. Non Alcoholics

ALT, AST, ALP and total bilirubin exhibited highly significant (P=.05, .01and .001 respectively) differences among the groups (Table-IV). The higher values of AST as compared to ALT were also recorded in alcoholics. Likewise, the ratio of AST/ALT in the studied groups was reflecting the progression of cirrhosis in preponderance alcoholics. The exposure duration/time in alcoholics also shed light in the development of alcoholic liver disease. The highest values of AST and ALT (138.40 IU/L and 62.60 IU/L) were observed in group D (11 Yrs and above Exposure time) followed by groups C (05-10 Yrs Exposure time) and B (01-04 Yrs Exposure time) as in comparison with that of non-alcoholics (Group A served as control). The same patterns were also noted in case of alkaline phosphatase (ALP) and total bilirubin levels between and within the studied groups (Table-IV).

Circulating Stress Biochemical Markers Profile of Alcoholics Vs Non Alcoholics

PARA-METERS	GROUPS	MEAN±SD	(n)	P VALUE
MDA	A	1.36±0.03	20	.000*
	B	7.78±1.70	4	
	C	8.28±1.68	41	
	D	8.45±1.63	5	
SOD	A	0.73±0.25	20	.016*
	B	0.06±0.05	4	
	C	0.12±0.10	41	
	D	0.30±0.22	5	
GSH	A	9.77±1.17	20	.000*
	B	2.24±0.94	4	
	C	2.04±0.85	41	
	D	2.16±0.97	5	
Catalase	A	4.27±0.73	20	.000*
	B	0.77±0.83	4	
	C	0.74±1.36	41	
	D	0.47±0.25	5	

Table-V. Stress Biomarkers Profiles of Alcoholics vs. Non Alcoholics

The consistent increasing trend in MDA levels (1.36, 7.78, 8.28 and 8.45 nmol/ml) were recorded in different groups (A-D) (Table-V). The consisting decreasing trends in case of Glutathione from groups A-C were recorded (9.77, 2.24 and 2.04 μg/dl) with a slight increase in group D (2.16 μg/dl). Catalase levels were also shows the consisting decreasing trend (4.27, 0.77, 0.74 and 0.47 μmol/mol of protein) in different studied groups (A-D) by the passage of exposure time on alcohol (Table-V). The lowest value (0.06 ng/ml) of SOD was recorded in group B (01-04 Yrs Exposure

time) and highest in case of non-alcoholics (0.73 ng/ml). An increasing trend from 0.13-0.30 ng/ml of SOD was recorded in groups C (05-10 Yrs Exposure time) and D (11 Yrs and above Exposure time) respectively demonstrating and stabilizing trend with the passage of exposure time on alcohol.

Lipid Profile of Alcoholics Vs Non Alcoholics

The results depicted in Table-VI reflecting that the lipid profile (TCh, Tg, LDL and HDL) of alcoholic versus non-alcoholics differed significantly (P=.05, .01and .001 respectively). In case of blood TCh and HDL levels a decreasing trend was recorded as compared to control between and within the studied groups. But reverse is true for Tg and LDL blood levels (Table 06). The consisting pattern of decreasing and increasing trends of blood TCh and Tg levels were recorded within the studied groups (A-D). The highest value (4.44 mmol/L) of TCh was recorded in group A (Non-alcoholics) followed by groups B (3.60 mmol/L), C (3.18 mmol/L) and D (2.72 mmol/L) respectively (Table-VI). But in case of Tg the highest value was recorded in group D (1.94 mmol/L) followed by groups C (1.85 mmol/L), B (1.81 mmol/L) and A (1.24 mmol/L) respectively. Inconsistent pattern of increasing and decreasing trends of blood LDL and HDL levels were recorded within the studied groups (A-D).

PARA-METERS	GROUPS	MEAN±SD	(n)	P VALUE
TCh	A	4.44±0.37	20	.000*
	B	3.60±0.41	4	
	C	3.18±0.49	41	
	D	2.72±0.56	5	
TG	A	1.24±0.15	20	.000*
	B	1.81±0.11	4	
	C	1.85±0.29	41	
	D	1.94±0.24	5	
LDL	A	2.31±0.15	20	.000*
	B	3.18±0.52	4	
	C	2.88±0.44	41	
	D	2.81±0.66	5	
HDL	A	1.73±0.17	20	.000*
	B	1.18±0.04	4	
	C	1.22±0.19	41	
	D	1.48±0.49	5	

Table-VI. Lipid Profiles of Alcoholics vs. Non Alcoholics

Significant levels < 0.05

DISCUSSION

Toxicity may be enhanced by malnutrition and malabsorption of nutrients and vitamins. In the development of alcohol-related pathology, certain bodily systems and circulating biochemical markers are markedly more vulnerable than others.

Hemoglobin (Hb) was observed to has direct and strong correlation (Table-VII) with RBC and GSH (r=.716** and r=.832** respectively) and was statistically significant (P<0.01). There is an increase of total bilirubin (TB) level of serum of moderate and heavy alcoholic patients, respectively (Table-IV). Creatinine was found to be as indirectly correlated (Table-VII) with Hb and RBC (r=-.769** and r=-.778** respectively; P<0.01), on the other hand, as directly strongly correlated with ALP and TB (r=.831** and r=.847** respectively; P<0.01). The bilirubin level in association with urea and creatinine may be used as markers in combination for ALD.

Hepatic enzymes leak into the circulation when hepatocytes or their cell membranes are damaged. Although these aminotransferases are sensitive indicators of liver cell damage, neither alone is an ideal marker. A very strong direct and indirect correlation (Table-VII) of ALP was found with TB, MDA and GSH (r=.950**, r=.929** and r=-.967**) and were statistically significant (P<0.01).

Alcohol abuse is one of the most causes of acute and chronic liver disease in worldwide. In western countries 50 percent of end stage liver diseases have alcohol as a major causative factor. The prognosis of liver cirrhosis is more than other common types of cancer for example breast, prostate and colon cancer. Unfortunately food and drug administration (FDA) has not yet designed any widely applicable drug therapy for alcoholic liver cirrhosis.[14] Most of the liver cancers are of epithelial cell origin but very rare can be of nonepithelial origin. Hepatocellular carcinoma is most common type occurring overall the world.

Long term intake of alcohol recognized as a major

cause of liver diseases specially hepatocellular carcinoma.[15] There are two mechanisms, direct (Genotoxic) and indirect mechanisms by which alcohol is involved in the development of hepatocellular carcinoma (HCC). Indirect mechanism is the most common pathway in the development of liver carcinogenesis in developed countries.[16] Alcoholic liver cirrhosis is a main globular health problem which results in fatty liver and inflammation and also leads to cirrhosis and hepatocellular carcinoma. It increases gut permeability which is associated with translocation of bacteria and their products. Lipopolysaccharide (LPS) is a major component of gram negative bacterial cell wall and by the action of parenchymal and nonparenchymal cells it is detoxified in the liver.[17] Some aspects of the relation between HCC and heavy alcohol drinking are still unsettled that is, the effect of consumption of alcoholic beverages, the age at which alcohol drinking start and the time while quitting. Hepatitis B and hepatitis C infection along with alcohol increase the risk of HCC and this is suggested by epidemiologic and pathological studies.[18,19]

Alcoholic liver diseases (ALD) are developing seriously in a large population of heavy drinkers. Alcoholic cirrhosis and alcoholic hepatitis appear to be predisposed by gender, diet, hereditary and occurrence of liver illness. Most of the liver damage is attributed to alcohol metabolism. Chronic alcohol consumption is a main factor and has a potent effect of liver disease on human health. ALD is a major and leading cause of liver cirrhosis throughout the world. Alcohol induced liver injury may also has some spectrums

	Hb	RBC	ALT	AST	ALP	TB	TCH	Tg	LDL	HDL	Urea	Creatinine	MDA	SOD	GSH	CAT
Hb	1	.716**	-.530**	-.665**	-.854**	-.847**	.676**	-.628**	-.484**	.524**	-.557**	-.769**	-.821**	.290*	.832**	.694**
		.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.015	.000	.000
RBC		1	-.452**	-.598**	-.841**	-.851**	.711**	-.638**	-.522**	.695**	-.470**	-.778**	-.756**	.282*	.858**	.754**
			.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.018	.000	.000
ALT			1	.727**	.585**	.559**	-.379**	.586**	.570**	-.256*	.406**	.457**	.545**	-.200	-.525**	-.378**
				.000	.000	.000	.001	.000	.000	.032	.000	.000	.000	.097	.000	.001
AST				1	.695**	.671**	-.643**	.543**	.412**	-.434**	.444**	.578**	.666**	-.209	-.665**	-.495**
					.000	.000	.000	.000	.000	.000	.000	.000	.000	.082	.000	.000
ALP					1	.950**	-.746**	.788**	.545**	-.630**	.667**	.831**	.929**	-.366**	-.967**	-.806**
						.000	.000	.000	.000	.000	.000	.000	.000	.002	.000	.000
TB						1	-.750**	.744**	.567**	-.654**	.616**	.847**	.899**	-.385**	-.940**	-.773**
							.000	.000	.000	.000	.000	.000	.000	.001	.000	.000
TCH							1	-.475**	-.262*	.564**	-.398**	-.630**	-.676**	.241*	.745**	.638**
								.000	.028	.000	.001	.000	.000	.045	.000	.000
Tg								1	.561**	-.454**	.505**	.591**	.744**	-.276*	-.760**	-.607**
									.000	.000	.000	.000	.000	.021	.000	.000
LDL									1	-.130	.363**	.552**	.532**	-.254*	-.544**	-.422**
										.283	.002	.000	.000	.034	.000	.000
HDL										1	-.323**	-.571**	-.530**	.212	.661**	.530**
											.006	.000	.000	.078	.000	.000
Urea											1	.516**	.658**	-.135	-.669**	-.529**
												.000	.000	.264	.000	.000
Creatinine												1	.800**	-.434**	-.835**	-.754**
													.000	.000	.000	.000
MDA													1	-.312**	-.909**	-.777**
														.008	.000	.000
SOD														1	.276*	.295*
															.021	.013
GSH															1	.793**
																.000
CAT																1

Table-VII. Pearson's Correlation Matrix of Circulating Biomarkers between Alcoholics and Non Alcoholics

*** Significant level (0.01)* *** Significant level (0.05)*

like steatosis, steatotohepatitis, cirrhosis and hepatocellular carcinoma. A mechanism that has a significant role in the development and progression of disease is epigenetic mechanism. This mechanism is involved in the parenchymal and nonparenchymal cells of liver. Its function is the initiation of inflammation and fatty liver. It also contribute towards hepatocytes necrosis and apoptosis.[20, 21]

In case of chronic and/or acute condition of autoimmune hepatitis, viral or drug-induced hepatitis ALD, the concentrations of aminotransferases in serum is moderately raised.[22] The diagnosis of some liver diseases is achieved by using the ratio of AST/ALT. The group of ALD with heavy intake of alcohol represented significant increase in AST/ALT ratio as compared to the group of ALD with lesser intake of alcohol. A necessary coenzyme referred as pyridoxal-5'-phosphate for both AST and ALT. The deficiency of this coenzyme is observed in ALD. Such deficiency leads to the decrease in hepatic ALT to a greater extent as compared to AST.[23,24] In the present study, the serum ALP level was significantly higher in group B (170%) group C (187%) and group D (189%) in comparison to non-alcoholics.

Plasma levels of MDA, SOD, catalase and GSH were expressed in Table-V. Statistically significant elevation was observed in MDA levels among the alcoholics as compared to the non-alcoholics. In comparison with group C and D, the decreased levels of MDA were observed in B group because chronic consumption of alcohol is associated with elevation in lipid peroxidation (LPO).[25] The pathogenesis of ALD has been associated with the peroxidation of polyunsaturated fatty acids (PUFA). GSH is responsible for the protection of cellular components from the damaging free radicals and also play an important role in redox balance. In comparison to group C and D the high alcohol intake leads to alcoholic liver disease (ALD). MDA was observed having very strong indirect correlation (Table VII) with GSH and catalase ($r=-.909^{**}$ and $r=-.777^{**}$ respectively, $P<0.01$). Decrease in hepatic GSH levels by the chronic consumption of alcohol induces oxidative stress.[22,25] Moreover, hepatic GSH has an important association with LPO due to its ability to bind with free radicals that may responsible for peroxidation.[26] Patients with alcoholic liver disease have lower hepatic GSH levels, which appear to be independent of nutritional status and probably reflect increased oxidative stress.[27] Several factors contribute to the fall in hepatic GSH level in alcoholic liver disease. A direct strong association (Table VII) between GSH and catalase was found ($r=.793^{**}$, $P<0.01$).

CONCLUSION:

All parameters in combinations may be useful indicator or may be good and reliable biochemical markers for identification and determination of severity of alcoholic liver diseases (ALD). The damaging of hepatocytes due to the consumption of alcohol disturbs almost all types of biochemical coordination in the biological system.

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"Contentment is the greatest wealth."

Shuja Tahir

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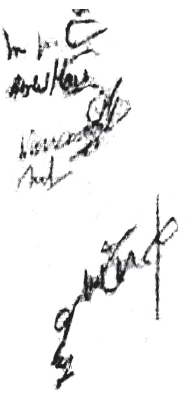
1	Dr. Muhammad Saeed Qureshi		
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