Research Article

Serum IL-8 as a prognostic factor in alcoholic liver disease patients with cognitive dysfunction

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Abstract

Introduction: Serum IL-8 levels are mediated by endotoxin release and activate neutrophil infiltration, a pivotal process in the pathogenesis of ALD. IL-8 levels also reflect the stage and severity of ALD and might serve as a predictor of survival in patients with alcoholic hepatitis. However, the role of IL-8 as a marker of liver disease and its association with cognitive changes in Alcoholic liver disease has not been clearly elucidated. We hypothesize that IL-8 levels can determine the stage and severity of alcoholic liver disease, and may serve as a prognostic marker in patients with alcohol related liver damage. **Material and Method:** A total of 68 cases and 50 age matched healthy controls were recruited. These patients were further categorized into 3 groups; fatty liver, alcoholic hepatitis and alcoholic cirrhosis. Enrolled patients were followed for 6 months. The study was approved by the institutional ethical committee. The alcohol withdrawal syndrome was assessed with the alcohol withdrawal scale (AWS) and Global cognitive functions were assessed periodically with Mini-Mental State Examination (MMSE). Serum levels of IL-8 were determined with an enzyme-linked immunosorbent assay (ELISA) provided by Diaclone. **Conclusion:** Serum IL-8 can be used as a marker to determine stages of alcoholic liver disease and correlates with cognitive dysfunction. Also high levels of IL-8 > 216 pg/ml were associated with poorer long term prognosis.

Key Word: Alcoholic liver disease; IL-8; prognosis.

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INTRODUCTION

Alcoholic liver disease (ALD) is a growing health problem and a major cause of morbidity and mortality worldwide. Alcohol consumption is responsible for 4% of the global burden of disease, and results in approximately 2.5 million deaths each year [1]. Alcohol consumption enhances endotoxin production and activates Kupffer cells[2, 3] which produce reactive oxygen species (ROS), cytolytic proteases, proinflammatory cytokines and chemokines[4,5,6] contributing to alcoholic hepatitis and cirrhosis in humans. This further leads to hepatic encephalopathy (HE) resulting in cognitive dysfunction, altered mood and motor in coordination[7]. Interleukin 8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells[8], airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles, the Weibel-Palade bodies. Interleukin-8 secretion is increased by oxidant stress, which thereby cause the recruitment of inflammatory cells and induces a further increase in oxidant stress mediators, making it a key parameter in localized inflammation[6] Serum IL-8 levels are mediated by endotoxin release and activated IL-8 mediates neutrophil infiltration, a pivotal process in the pathogenesis of ALD[8, 9, 10]. IL-8 levels also reflect the stage and severity of ALD and might serve as a predictor of survival in patients with alcoholic hepatitis. IL-8 appears to be an important factor in liver pathology in patients with ALD, especially in the development of the inflammatory process[11, 12]. However the role of IL-8 as a marker of liver disease and its association with cognitive changes in Alcoholic liver disease has not been clearly elucidated. We hypothesize that IL-8 levels can determine the stage and severity of

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alcoholic liver disease, and may serve as a prognostic marker in patients with alcohol related liver damage. We compared the sensitivity of IL-8 with other traditional liver enzymes and ascertained its role in cognitive changes and for prediction of long term mortality.

METHODS AND MATERIALS

The study was a hospital based case control studyconducted in Department of Biochemistry and Department of Psychiatry, Padmashree Dr D.Y Patil Hospital and Research Centre, Nerul Navi Mumbai. A total of 68 adult male patients, consecutively transferred to inpatient detoxification center were recruited for the study after confirmation of alcoholic liver disease on the basis of clinical findings and by USG studies of liver. These 68 patients were further categorized on the basis of clinical findings into 3 groups; fatty liver (Group A), alcoholic hepatitis (Group B) and alcoholic cirrhosis (Group C). Enrolled patients were followed for 6 months. The control group comprising of 50 age matched healthy individuals were recruited from volunteers and healthy persons accompanying the patients in the general outpatient department (OPD). Informed consent was obtained from all subjects before the collection of information. The study was approved by the institutional ethical committee.

Clinical History

Detailed history including amount, duration, type of alcohol consumption in the form of whisky, rum, wine, vodka was taken. Alcohol dependency was enquired in the form of CAGE questionnaire. All 68 patients fulfilled the ICD-10 (The Tenth Revision of the International Classification of Diseases and Health Problems) WHO 1992 criteria for alcohol dependence. The alcohol withdrawal syndrome was assessed with the alcohol withdrawal scale (AWS).The maximum AWS-score was taken as indicator of the severity of AWS[15]. Global cognitive functions were assessed periodically with Mini-Mental State Examination (MMSE).A MMSE score of 23 is taken as cut-off as it is the most widely accepted and frequently used. Scores of 23 or lower indicates the presence of cognitive impairment[14]. Of 118 patients enrolled in the study, 66 patients (97.0%) completed the full 6 months of follow-up, 7 patients (10%) died. Alcoholic liver disease was classified by subtype.

Biochemical analysis

On admission of patient ten milliliter of venous blood was collected using all aseptic method. After clotting the samples were immediately centrifuged at 3000 rpm for 10 minutes to separate serum which was stored at -80°C until further analysis. The samples for AST, ALT and GGT were analyzed as follows: The Aminotransferases (AST, ALT) method is an adaptation of methodology recommended by International federation of clinical chemistry (IFCC) without pyridoxal phosphate (P-5'-P) Kinetic U.V using automated laboratory procedures (VITALAB SELECTRA E). The Gamma-glutamyltransferase (GGT) method is an adaptation of methodology recommended by using International federation of clinical chemistry (IFCC) methods using automated laboratory procedures (VITALAB SELECTRA E). The method uses the substrate L-gamma glutamyl- 3-carboxy- 4-nitroanilide with glycylglycine. Serum levels of IL-8 were determined with an enzyme-linked immunosorbent assay (ELISA) provided by Diaclone Research.

Statistical Analysis

All data were fed on excel spreadsheet and statistical analyses were made using SPSS version 17.0.We used the student t test to compare the means. The level of significance was chosen to be $p<0.05$.Categorical data were summarized as frequencies and percentages. Continuous data were summarized as median (minimum, maximum). The relationship between inflammatory markers and outcome measures was determined using Spearman rank correlation. We used *the X^2 test with Yates* correction. Chi square and Fisher's exact test when appropriate and the Odds ratio (OR) along with 95% CI to compare proportions between the groups. We used Mann-Whitney/Wilcoxon Two-Sample Test (Kruskal-Wallis test for two groups). Receiving operating characteristics tool has been used to find the diagnostic performance of study parameters. The Receiving operating characteristics (ROC) is a graphical representation of relationship between sensitivity and specificity of a laboratory tests. Kaplan Meier survival analysis was done to see association between IL-8 levels and mortality.

RESULT

The clinical characteristics of the study group are shown in (Table 1). A total of 68 males were enrolled in the study. The mean age of study population was 41.68±9.84 years. The clinical characteristics of the study group are shown in Table 1. The study groups were age- matched (p. 005).

Table 1: Descriptive Statistics

Parameters	Cases 68	Controls 50
Age	41.68±9.84	34.90±8.57
Bmi	22.23±2.91	20.82±1.96
Alcohol consumption Type	Country liquor	-------
Duration	5-20 years	-------
Amount	115.8±44.41	-------

All controls were healthy with normal routine laboratory investigations and as was expected serum values of AST, ALT, AST/ALT, GGT and IL8 were found significantly more often in cases than in controls (Table 2), p<. 0001).

Table 2: Serum levels of AST, ALT, Deritis ratio AST/ALT, GGT and IL-8 in all cases [68] and controls [50]

.	Cases (n68) Mean	Controls (n50) Mean	P value
AST	118.51±79.9	20.3±5.22	0.001*
ALT	73±37.8	68.51±19.3	0.001*
AST/ALT	1.82±0.9	0.779 ±0.13	0.001*

GGT	137.62±14.1	25.48±10.8	0.001*
IL-8	216.4± 31.4	20.±6.0	0.001*

*Denotes statistically significant

(Table 3) shows the levels of various parameters in different groups of ALD. The IL-8 levels were highest in Alcoholic cirrhosis followed by alcoholic hepatitis and fatty liver The GGT levels were highest in Alcoholic hepatitis followed by alcoholic cirrhosis and fatty liver. On the contrary the other parameters did not follow any such patterns.

Table 3: Serum levels of AST, ALT, Deritis ratio AST/ALT, GGT and IL-8 in fatty liver, hepatitis, cirrhosis

Parameters	Fatty liver [N=23] Mean±SD	Hepatitis [N=21] Mean±SD	Cirrhosis [N=24] Mean±SD
AST (IU/L)	95.73±61.1 NS	130.7±66.35* P<0.001	135.8±85.8* P<0.001
ALT (IU/L)	61±30. NS	83.0±12.0* P<0.001	82.0±32.0 NS
AST/ALT	1.64±0.6* P<0.001	1.96±1.48* P<0.001	1.79±.99* P<0.001
GGTIU/L	60.99±36.7* P<0.001	227.08±170.5* P<0.001	143.74±105.4* P<0.001
IL-8pg/ ml	39±21.6* P<0.001	202.7±181* P<0.001	414.5±364* P<0.001

*Denotes statistically significant.

[N.B.-Results of every parameter of every type of liver disease are compared to that found among controls]

The MMSE levels were lowest in Alcoholic cirrhosis followed by alcoholic hepatitis and Fatty liver. The AWS levels were highest alcoholic cirrhosis followed by Alcoholic hepatitis followed and fatty liver (Table 4).

Table 4: MMSE and AWS scores in different stages of ALD

Parameters	Fatty liver [23]	Hepatitis [21]	Cirrhosis[24]	P value
MMSE	19±3.5	13±4.4	11±5.4	0.001*
AWS	12±3	14.15±3.77	16±2.7	0.000*

*Denotes statistically significant.

We found significant correlation between alcohol consumption and cognitive dysfunction measured with the help of cognitive scales MMSE and AWS (Table 5). Patients with MMSE values < mean value 14.16 was considered to have bad MMSE scores and patients with AWS mean >13.50 were grouped as bad AWS. It was observed that large group of patients with mean value of alcohol consumption more than 115.89 gram/day (mean value) showed poor MMSE results 14(63.6%) with the (odds ratio [OR] 2.45; 95% confidence interval [CI] 0.86-6.93; P = 0.048) compared to patients with low alcohol consumption 8(36.4%) who showed good MMSE. Also in patients with heavy drinking, 15 patients (68.2%) with the (odds ratio [OR] 2.53; 95% confidence interval [CI] 0.87-7.32; P=0.045) developed severe alcohol withdrawal syndrome AWS (seizures, disordered perceptions, or delirium).

Table 5: Effect of alcohol consumption Gm/Day on MMSE and AWS

Alcohol consumption GM/DAY	MMSE Good no of cases (%)	MMSE Bad no of Cases (%)	OR (95%CI)	P value*	AWS Good no of cases (%)	AWS Bad no of Cases (%)	OR (95% CI)	P value*
>115.89 g/day	8(36.4)	14(63.6)	2.45(0.86-6.93)	0.048	7(31.8)	15(68.2)	2.53(0.87-7.32)	0.045
<115.89 g/day	28(58.3)	20(41.7)			26(54.2)	22(45.8)		

*Denotes statistically significant, OR, odds ratio.

We also studied correlation between liver enzymes, IL-8 and cognitive scales MMSE and A WS. IL-8 showed significant correlation with MMSE (p<0.001) and AWS. (Table-6)

Table 6: correlation between liver enzymes, IL-8 and cognitive scales

Parameters	Correlation coefficient MMSE	P value	Correlation coefficient AWS	P value	Parameters
AST	-0.21	0.07	0.06	0.62	AST
ALT	-0.10	0.38	-0.06	0.57	ALT
AST/ALT	-0.12	0.29	0.101	0.40	AST/ALT
GGT	-0.23	0.050	0.008	0.94	GGT

IL-8	-0.84	0.000*	0.75	0.00*	IL-8

*Denotes statistically significant

In this study, IL-8 (29pg/ml) seems to be a good inflammatory marker in patients of alcoholic liver disease for which area under the ROC curve was 0.929, and 90% sensitivity and 92 % specificity makes the test as excellent test. GGT (>50U/L) seems to be a good marker in patients of alcoholic liver disease area under the curve was 0.867, and 70% sensitivity and 98 % specificity.AST (> 37U/L), ALT(>65U/L) and AST/ALT (> 1) had area under curve 0.999, 0.941 and 0.998.(figure 1)

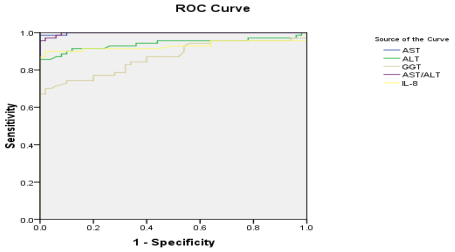

Figure 1: Diagonal segments are produced by ties

The mean levels of serum IL-8 levels were 216.4± 31.4. As shown in Figure 2, values of IL-8 above this were significantly associated with increased mortality (p = 0.000, log rank test)

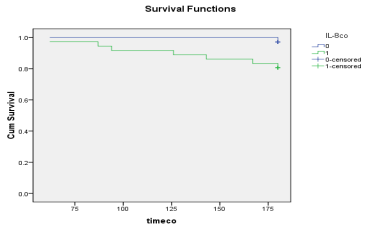

Figure 2: Kaplan Meir survival curve

DISCUSSION

Inflammation plays a crucial role in the pathogenesis of alcoholic liver disease. There is interplay of adducts, proinflammatory cytokines and chemo attractant chemokines culminating in liver cirrhosis. Hence, the role of inflammation cannot be understated. In this study, traditional markers GGT, AST, ALT, AST/ALT ratio were compared with interleukin-8 in assessing the sensitivities and specificities of these markers in alcoholic liver disease. We also assessed the association Of IL-8 and cognitive dysfunction and further evaluated its role in predicting long term prognosis of ALD. In our study Serum IL-8 levels were found to be significantly higher in alcoholic cirrhosis compared to hepatitis and fatty liver indicating role of inflammation in development of alcoholic liver disease. IL-8 demonstrated good sensitivity and specificity as a marker of ALD. IL8 levels > 29pg/ml was associated with increased long term mortality. Our study included the alcoholics who consumed mean alcohol of 115.89 gm/day for duration of minimum 5 to maximum 20 years thus increasing the risk and development of alcoholic liver disease. These findings were supported by Savolainen VT *et al* and Jørgensen *Et al* which established that the incidence of both bridging fibrosis and liver cirrhosis increased significantly only when daily intake of ethanol exceeded 80 g[16,17]**.** In our study mean levels of AST and ALT concentrations in Alcoholic liver disease was significantly higher as compared to controls and difference in levels between controls and cases is statistically significant (P<0.001). The increase of AST and ALT concentration in alcoholic liver disease was in accordance with other study[18,19]. Mean of AST/ ALT concentrations in our study was significantly higher as compared to controls with the ratio being 1.94 for hepatitis and 1.85 for cirrhosis consistent with the study of Cohen JA *et al*, Majhi *et al* [20, 21].. Peter C Sharpe showed that AST/ALT ratio of more than 1.5 strongly suggests and ratio>2.0 is highly indicative of alcohol induced liver disease and is elevated because of existing mitochondrial damage [22]. Mean GGT concentrations in our study was also significantly higher in cases as compared to controls, highest level being found in alcoholic which is also supported by findings of Gogoi *et al* and Tyagi *et al* [23].. Serum GGT is a biliary canalicular enzyme induced by alcohol, and released into circulation in response to acute hepatocellular injury and excessive alcohol consumption. Increased serum GGT is widely used as a marker of alcohol abuse [24, 25]. The ROC curve analysis in alcoholics in our study for GGT was found to be larger with low sensitivity and high specificity which was in discordance with findings of Pekka Sillanaukee who showed low specificity for alcohol abuse[26]. The sensitivity and specificity of GGT in our study was almost similar to the study done by other investigators[27]. Various clinical studies have shown that IL-8 is an independent prognostic marker for alcoholic Liver disease and the circulating levels might reflect stage and severity of ALD)[28] In the present study the mean value of IL-8 in Alcoholic liver disease was 216.4± 31.4 which was significantly higher as compared to controls and difference in levels between controls and cases is statistically significant (*P*<0.001). Highly elevated IL-8 levels were observed in alcoholic liver cirrhosis with *P*value<0.05.Study by Swiatkowska-Stodulska R etal, Kawaratani *et al* and Huang YS*et al* in Patients of ALD showed highly elevated levels of Serum IL-8 in patients with Alcoholic Hepatitis and in contrast IL-8 was only moderately elevated in alcoholic cirrhosis patients or alcoholics. This is linked to neutrophil infiltration. [9,12,29]. According to the findings by Hartmut Jaeschke, clear evidence for a neutrophil-induced injury in alcoholic hepatitis is uncertain[30]. This difference in the findings can be due to genetic predisposition, cultural and ethnic differences. However in our findings significantly high IL-8 levels were observed in alcoholic liver cirrhosis thus indicating that monocytes/macrophages, and not neutrophils, appear to be main responders to IL-8 in liver fibrosis/cirrhosis via

CXCR1 which was in accordance to the study by Zimmermann HW *et al* who established that IL-8 serum levels were significantly increased in chronic liver disease patients (CLD) patients, especially in end-stage Cirrhosis [31]. Increased of several serum cytokines levels has also been reported in alcoholic liver cirrhosis by Daniluk *et al* Deviere *et al* [32,33]. Observations by Daniluk *et al*[33] strongly suggests that IL8 is a reliable diagnostic parameter for evaluating the severity of liver damage. Study by Craig J. McClain, M.D.*et al* indicated the importance of collagen in healing; excess collagen deposition is a hallmark of cirrhosis[34]. Abraham P Bautista established that exacerbated production of the chemokines during chronic alcohol intoxication that may perpetuate persistent lymphocytic inflammation in the liver[35]. Chronic alcoholism can increase gut permeability to endotoxins and impair the reticuloendothelial function of the liver thus increasing plasma endotoxin concentration. Endotoxin is a major stimulus for the production of cytokines like TNF-alpha, precursor of IL-8. IL-8 in its turn mediates hepatic neutrophil infiltration and hepatic injury (Bode *et al*., 1987)[36]. Fulton showed Cytokines affect the brain and likely contribute to changes in the central nervous System that contribute to long-term changes in behavior and neurodegeneration[37] Long-term alcohol misuse causes alcohol related liver disease thus resulting in brain damage and dysfunction ranging from mild cognitive deficits, which are relatively common, to full-blown Korsakoff's psychosis. Wernicke's encephalopathy, caused by acute CNS thiamine deficiency [38] Participants were categorized into normal cognitive and cognitively impaired groups by education-adjusted MMSE cut-off scores. In present study the scores were found to be low in alcoholic liver cirrhosis compared to hepatitis and fatty liver. *P* value<0.001.The average daily alcohol consumption in the cognitively impaired group was significantly higher. [Mean (SD): 115.82±44.41grams per day]. It was observed as the alcohol consumption exceeded the mean value risk of cognitive impairment proportionately increased as assessed by MMSE (OR = 2.45, 95%CI = 0.86-6.93) and AWS (OR = 2.53, 95%CI = 0.87-7.32). Chan *et al* established in their studies that heavy alcohol consumption is associated with an increased risk of cognitive impairment while light to moderate alcohol consumption is associated with reduced risk which was in accordance with our study[39]. Various clinical studies have established that IL-8 is an independent prognostic marker for alcoholic liver disease and the circulating levels correlate with progression of disease. Our study showed significant elevation of Serum IL-8 in patients of alcoholic liver cirrhosis. significant correlation between IL-8 and MMSE and AWS with *P* value <0.001 was established in this study thus indicating that IL-8 is strongly activated in alcohol related liver cirrhosis and they in turn affect the brain and likely to cause changes in the central nervous system that contribute to long-term changes in behavior and neurodegeneration [37]. In this study, IL-8 (29pg/ml) behaved as a good inflammatory marker in patients of alcoholic liver disease. Receiving operating characteristic (ROC) analysis curves and the corresponding area under the curve were calculated for providing the accuracy of the cytokines in differentiating between the different groups under consideration. Sensitivity (i.e., true positive rate), specificity (i.e., true negative rate), positive predictive value, negative predictive value and cutoff values showing the best equilibrium between sensitivity and specificity were evaluated. Analysis of IL-8 by ROC curves (0.929) revealed satisfactory values regarding sensitivity (90%) and specificity (92%) at a cutoff value of ≥ 216 pg/ml. We found that increased serum levels IL-8 was above the mean value of 216.4± 31.4pg/ml were associated with a poorer outcome and increased mortality at 6 months. Our results were in accordance with Huang *et al* who demonstrated that higher serum IL8 levels correlated with higher mortality rate in Chinese population[29]. From the study it was concluded that estimation of serum IL-8 is more informative in detecting alcoholism if it is used along with other biochemical parameters like GGT, ALT, and AST. Also determination of serum IL-8 in different stages of alcoholic liver disease will be a useful diagnostic tool if used judiciously and correlated with MMSE and AWS. Also high levels of IL-8 were associated with poorer long term prognosis. This signifies the progression of the disease to cognitive impairment and assessment of alcohol withdrawal syndrome respectively to indicate

1. Effective management of withdrawal in its early stage
2. Reduce or prevent progression of disease

Thus it concludes that IL-8 not only identifies the decrease in MMSE status among liver diseases, but also the progression of liver disease among alcoholics.

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