



Research Article

The Study of TNF Alpha and Interleukin 6 (IL 6) in Cases of Cirrhosis and Its Correlation with Hepatic and Renal Impairment with Subacute Bacterial Peritonitis

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Abstract

Background: We studied TNF alpha and IL 6 level in cirrhotic patient with SABP and their correlation to hepatic and renal impairment and mortality.

Methods: This was a prospective correlational study and 100 consecutive patients with cirrhosis and ascites were studied. Serum and Ascitic Fluid were collected at Study entry and 48 hours later. Fluid was sent for cell count, biochemical assay, culture and sensitivity. Assays for TNF alpha and IL 6 in the serum and ascitic fluid were performed with ELISA using manufacturer's instructions.

Results: Out of 100 patients –57 were in group I (Group with SABP) and 43 were in group II (Group without SABP). Out of 57 patients of group I, 25 were in Group Ia (with Renal Impairment) and 32 were in Group Ib (without Renal Impairment). Group Ia, all patients belonged to Child Score C. The level of cytokines in serum and ascitic fluid correlated significantly in this group and also significantly correlated with mortality.

Conclusion: In group Ia level of TNF alpha and IL 6 rise was statistically significant and significantly correlated with mortality as all of the patients of this group had CTP scoring of Child C. The cytokines production enhances liver cell injury leading to renal impairment and levels correlated well with poor prognosis and mortality seen in SABP in cirrhotic patients with renal impairment. We conclude, that measurement of the cytokines levels is a non-invasive simple method for diagnosis and prognostication in cirrhosis patients.

Keywords: Cytokines, cirrhosis and subacute bacterial peritonitis, TNF alpha and IL6 in cirrhosis with renal failure, subacute bacterial peritonitis with renal failure.

Introduction

Cirrhosis is defined as a disease process in the liver characterized by the development of extensive fibrosis and replacement of normal liver architecture by structurally abnormal nodules or fibrotic tissue^[1].

SABP is a severe complication of cirrhotic patient with ascites. SABP is associated with production of inflammatory mediators. Cytokines especially Interleukin 6 (IL-6) and TNF alpha are probably the most important mediators and patient with chronic liver disease responds to sepsis with greater and

long lasting increase in the circulating levels of these cytokines. The rise in level of cytokines are directly related to mortality. The levels are highest in patient with renal impairment because of marked activation of Renin Angiotensin System specially in alcoholic liver disease.

Renal impairment (RI) in the course of SABP is a frequent event in cirrhotic patients with ascites and constitutes the most important predictor of hospital survival. These cytokine levels were highest in patients who have renal impairment and its levels positively correlated with liver disease progression and served as an independent predictor of hospital mortality.

Methods

Study Subjects: The study was a prospective correlational study which included patients with cirrhosis and ascites who were suspected to have Spontaneous Bacterial Peritonitis (SABP) attending the Outpatient Department and Indoor units of Department of Medicine from the year January 2015 to June 2016 for a period of 18 months.

Study Methodology: A detailed history, physical examination and investigations were carried out. Diagnosis of cirrhosis was made by clinical, biochemical findings (low serum albumin, AST: ALT>1) and imaging findings (USG Abdomen – heterogenous echotexture of liver with irregular outline, altered liver size depending on aetiology, portosystemic collaterals) [2].

Diagnosis of ascites was based on clinical findings and imaging findings. The diagnosis of SABP was based on ascitic cytology showing >250 cells of PMN [3] and or culture positivity with absence of findings of secondary peritonitis. Cytokine levels were measured on day 0 and day 2 using ELISA Kits in serum and ascitic fluid.

Impaired renal function was defined as serum creatinine values >1.5 mg/dl [4]. Impaired hepatic function was defined by decreased serum albumin, increased serum bilirubin and increased PT/INR.

In our study patients were divided into two groups – Group I - with SABP and Group II - without SABP. The group I with SABP was further divided into

Group Ia - SABP with Renal Impairment (RI) and Group Ib - SABP without Renal Impairment.

Out of the 100 patients – 57 belonged to the group with SABP (I), of which 25 had RI (Ia) and 32 had no RI (Ib) and 43 belonged to without SABP group (Group II).

Collection of Samples: Serum and Ascitic Fluid were collected at Study entry (before the initiation of antibiotic treatment) and 48 hrs later. Ascitic Fluid was sent to lab for cell count, biochemical assay, culture and sensitivity. Assays for TNF alpha and IL 6 in the serum and ascitic fluid were performed with ELISA using manufacturer's instructions.

Statistical Method

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean \pm SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected, then non parametric test was used.

Statistical tests were applied as follows-

1. Quantitative variables were compared using Unpaired t-test/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups and Anova/Kruskal Wallis test (for non-parametric data) between more than two groups.
2. Qualitative variables were correlated using Chi-Square test /Fisher's exact test.
3. Pearson correlation coefficient/ Spearmen correlation coefficient (for non-parametric data) was used to find association between two quantitative variables.

A p value of <0.05 was considered statistically significant.

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

Results

Serum bilirubin, AST, ALT and INR levels were found to be significantly raised in the group Ia than the other two groups, while serum albumin, had no statistical significance among the three groups.

All patients of group Ia belonged to Child score C, indicating that the patients with SABP with renal impairment had a significantly more severe form of cirrhosis and all of them had significantly high levels of TNF alpha and IL 6 in serum and ascitic fluid.

Serum Creatinine levels were found to be significantly raised in the group Ia than the other two groups.

Treatment with antibiotics for 48 hours, results in significant decrease in TNF alpha and IL 6 level in serum and ascitic fluid.

Serum and Ascitic fluid TNF alpha and IL 6 levels were found to significantly correlate with serum ALT, AST and bilirubin and serum creatinine with a p value of <0.0005.

Higher level of cytokines in serum and ascitic fluid suggested higher renal impairment. Cytokine level has significant correlation with mortality in patient with SABP.

However, it was found in our study that serum albumin levels had no statistical significance among the three groups. There was also a negative correlation between serum and ascitic fluid cytokine levels with serum albumin and coagulopathy in our study.

Discussion

Subacute Bacterial Peritonitis (SABP) is considered as a complication of cirrhotic patients with ascites characterized by spontaneous bacterial infection of previously sterile ascitic fluid in cirrhotic patients, without any apparent surgically treatable intra-abdominal source of infection. It is a common and potentially fatal complication of cirrhosis and ascites.

SABP is thought to appear as a consequence of impaired defence mechanism against infection seen in cirrhotic patients. Cytokines may regulate immune responses and high plasma cytokine levels have been demonstrated in patients with infectious disease. The cytokines TNF alpha and IL 6 are produced in large amounts during the inflammation by mainly monocytes and macrophages. They are

considered as a response to injury since they are not produced by normal tissue.

Patients with liver cirrhosis respond to sepsis with a greater and long lasting increase in the circulatory levels of IL 6 and TNF alpha, than the patients without cirrhosis. Cytokines particularly TNF alpha and IL 6 are probably the most important mediators of sepsis.

Renal impairment in the course of SABP is a frequent event in cirrhotic patients with ascites and predictor of hospital survival. These patients develop circulatory dysfunction due to Nitric Oxide (NO) production leading to splanchnic and arteriolar vasodilation, whose induction occurs by synergistic action of different cytokines. This in turn leads to decreased effective blood volume, hypotension, activation of Renin Angiotensin Aldosterone System (RAAS).

Group Wise Distribution of Patients – Results

In our study out of 100 patients studied, Group I had 57 (57%) patients [Group Ia - 25(43.8%), Group Ib - 32 (56.14%)] and Group II had 43 (43%) patients.

Correlation of Serum TNF Alpha and IL 6 at the time of Admission

Serum TNF alpha had median of 286.2, 156.25, 63.9 (276.6 ± 32.58 , 150.49 ± 27.93 , 66.94 ± 11.34) pg/ml and Serum IL 6 had median of 283.2, 151.5, 67.4 (277.22 ± 35.09 , 148.67 ± 24.75 , 68.14 ± 11.07) among the three groups Ia, Ib and II respectively and on applying ANOVA test, p value of < 0.0005 showed that serum IL 6 and TNF alpha levels were significantly correlated with the 3 groups.

Ascitic TNF alpha had median of 291.4, 168.3, 79.2 (292.08 ± 34.27 , 164.98 ± 23.4 , 78.87 ± 15.72) and Ascitic IL 6 had median of 299.4, 165.8, 84.6 (302.73 ± 34.9 , 163.95 ± 20.49 , 82.46 ± 9.18) among the three groups Ia, Ib and II respectively and on applying ANOVA test, p value of < 0.0005 showed that ascitic fluid IL 6 and TNF alpha levels were significantly correlated with the 3 groups. This showed that the serum TNF alpha and IL 6 and Ascitic Fluid TNF alpha and IL 6 levels were significantly raised in the group Ia.

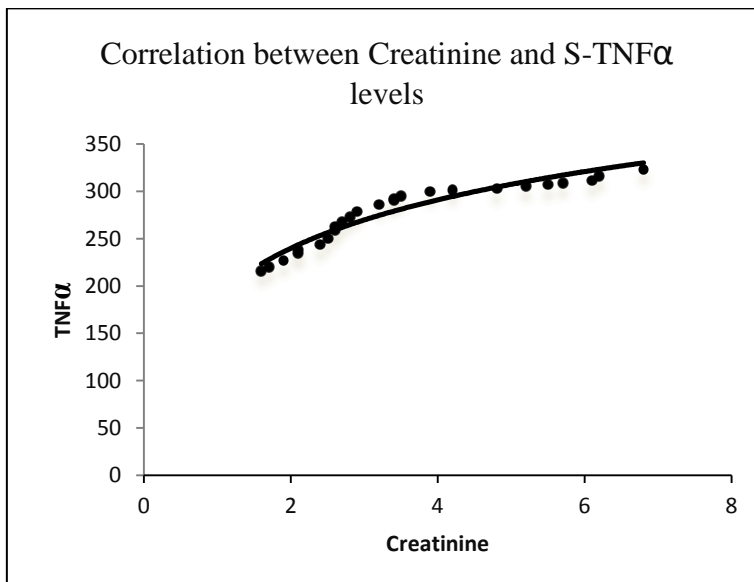


Fig.1- Correlation between Creatinine and serum TNF- α at day 0

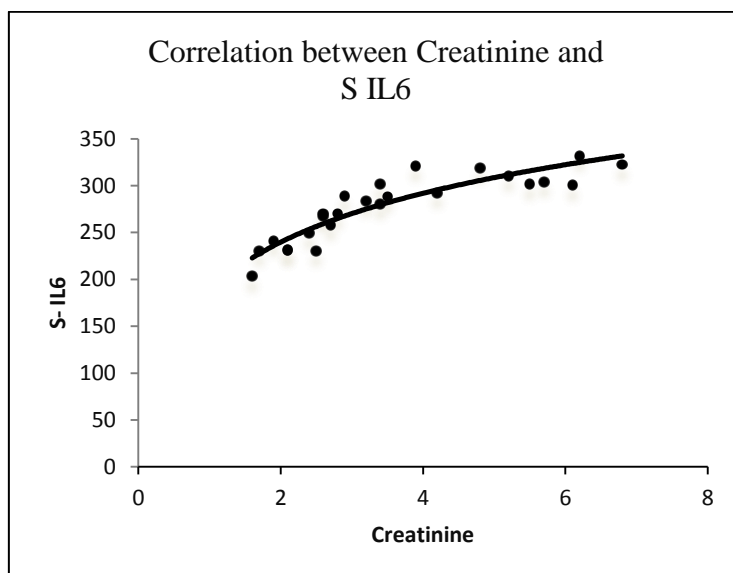


Fig.2-Correlation between Creatinine and IL-6 at day 0

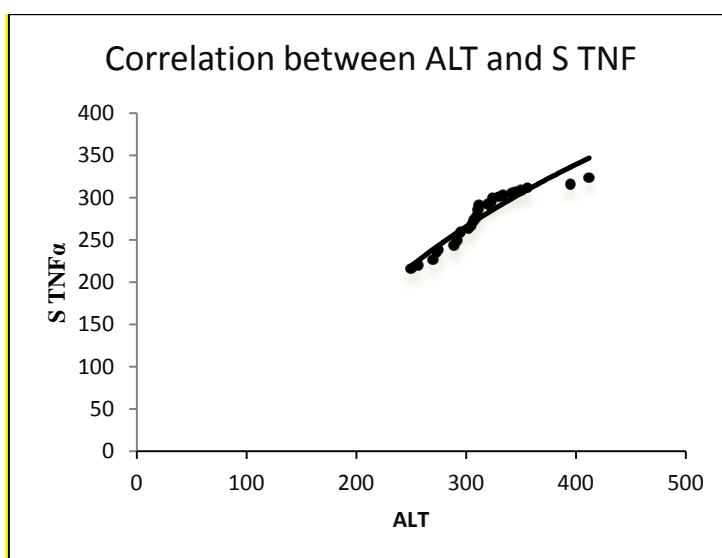


Fig.3- Correlation between ALT and serum TNF- α at day 0

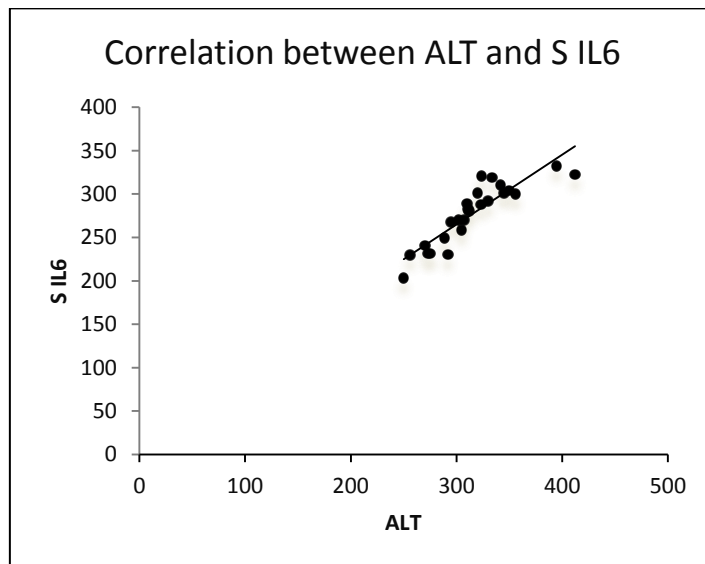


Fig.4- Correlation between ALT and serum IL-6 at day 0

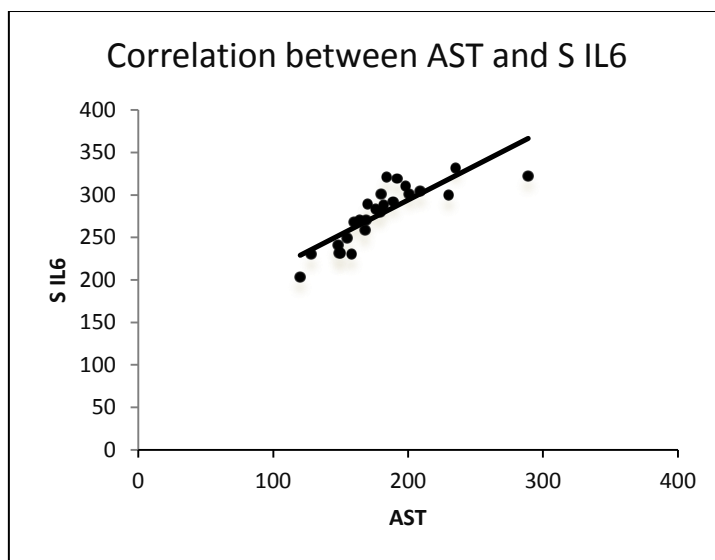


Fig.5-Correlation between AST and serum IL-6 at day 0

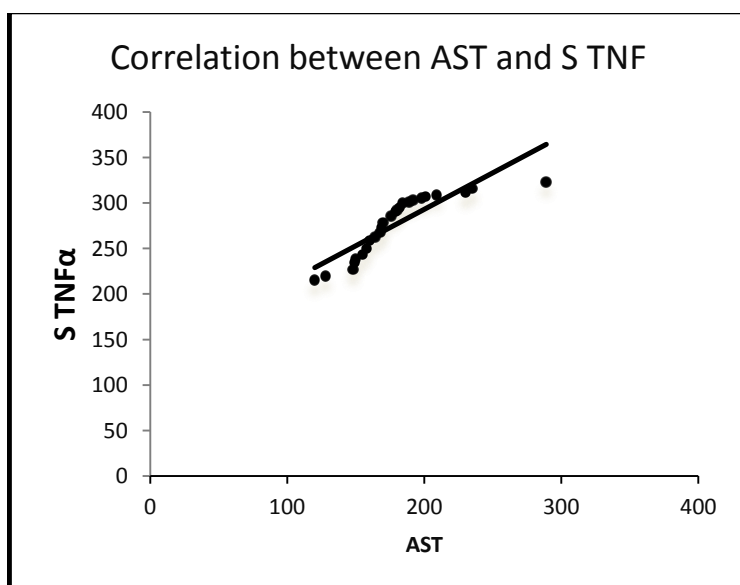


Fig.6-Correlation between AST and serum TNF-α at day 0

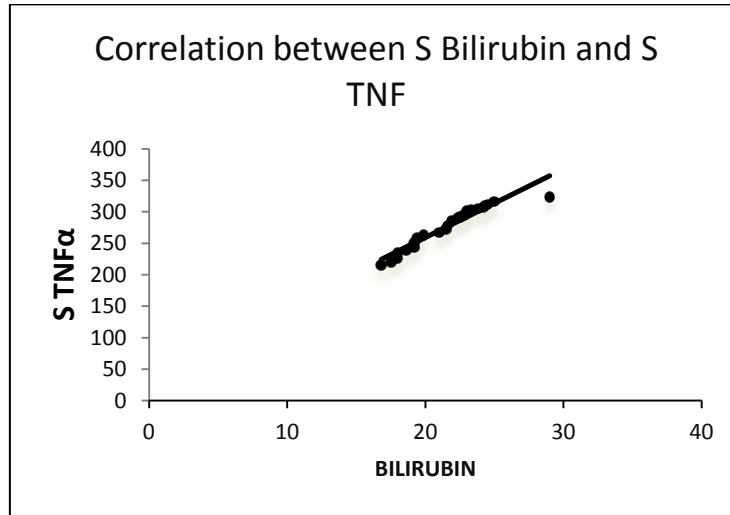


Fig.7- Correlation between S.bilirubin and serum TNF-α at day 0

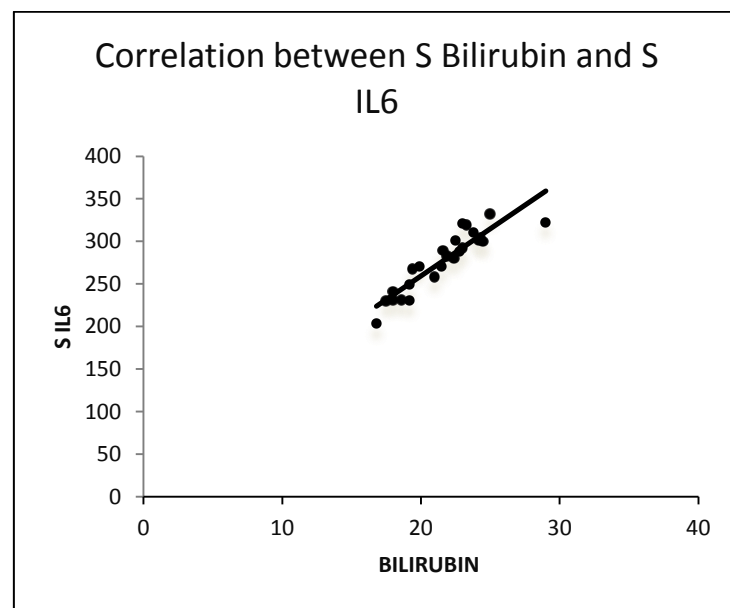


Fig.8-Correlation between S.bilirubin and IL-6 at day 0

Correlation of Serum TNF Alpha and IL 6 on Day 2–

Serum TNF alpha had median of 40.3, 25.75, 27 and Serum IL 6 had median of 42.1, 25.8, 23.4 among the three groups Ia, Ib and II respectively and on applying ANOVA test, p value of < 0.0005 showed that serum TNF alpha levels and IL 6 were significantly correlated with the 3 groups.

Ascitic TNF alpha had median of 40.3, 25.8, 24.6 and Ascitic IL 6 had median of 38.7, 27.05, 31.4 among the three groups Ia, Ib and II respectively and on applying ANOVA test, p value of < 0.0005 showed that ascitic fluid IL 6 and TNF alpha levels were significantly correlated with the 3 groups.

Percentage Change in TNF Alpha and IL 6 from Day 0 to Day 2

Percentage change in serum TNF alpha levels had a median of 85.06, 82.63, 58.99 among the group Ia, Ib and II and Percentage change in serum IL 6 levels had a median of 84.34, 83.17, 64.44 among the group Ia, Ib and II respectively and the p value so obtained on applying ANOVA test was <0.0005 showed that the fall in IL 6 and TNF alpha levels from day 0 to day 2 were significant.

Percentage change in ascitic fluid TNF alpha levels had a median of 85.68, 84.44, 70.88 among the group Ia, Ib and II and Percentage change in ascitic fluid IL 6 levels had a median of 88.48, 83.5, 63.29 among the group Ia, Ib and II respectively and the p

value so obtained on applying ANOVA test was <0.0005 showed that the fall in IL 6 levels and TNF alpha from day 0 to day 2 were significant.

This showed that there was a significant decline in the levels of TNF alpha and IL 6 both in serum and ascitic fluid from day 0 to day 2 after the patient were treated with antibiotic.

Correlation of Serum and Ascitic Fluid TNF Alpha and IL 6 with Liver Function Test –

In Group Ia –

Serum and Ascitic fluid TNF alpha and IL 6 levels on applying Pearson correlation were found to significantly correlate with serum ALT, AST, bilirubin and a p value <0.0005 showed that the correlation is significant.

However, there was a negative correlation of serum and ascitic fluid TNF alpha and IL 6 with serum albumin and INR and there was no statistically significant correlation between the cytokine levels and serum albumin and coagulopathy.

Correlation of Serum and Ascitic Fluid TNF Alpha and IL 6 with Kidney Function Test –

In Group IA –

Serum and Ascitic fluid TNF alpha and IL 6 levels on applying Pearson correlation were found to significantly correlate with serum creatinine with a p value of <0.0005 .

It was also found in our study that Serum and Ascitic fluid TNF alpha and IL 6 levels were higher in the group Ia when compared to group Ib, thereby suggesting that renal impairment occurs in patients with highest concentration of cytokines.

In our study renal impairment correlated well with the increased levels of TNF alpha and IL 6 at the time of diagnosis of SABP, and this reflected there is a relationship between the degree of inflammatory response at infection diagnosis and the development of SABP with RI and our study also shows that higher the creatinine, the higher were the levels of serum and ascitic fluid TNF alpha and IL 6.

Correlation of Child Pugh Score

Out of 100 patients, 67 belonged to Child Pugh C (67%) and 33 belonged to Child Pugh B (33%).

Among the 67 patients of Child Pugh C – 25 (100%) patients were found in the group Ia, 27 (84%) patients were found in the Ib group and 15 (34%) patients were found in group II. Out of the 33 patients of Child Pugh B - 65% patients (28) belonged to group II, 15% (5) belonged to the group Ib and 0% belonged to the group Ia.

The above data found in our study showed that in the Group Ia - 100% of the patients belonged to Child score C, showing that the patients with SABP with renal impairment had a significantly higher Child Pugh Score. While in the Group Ib - 84% patients were in Child C and 15% in Child B. In the Group II - 15% patients were in Child C and 65% in Child B.

Our study reported a significant increase of serum TNF alpha and IL 6 according the severity of liver disease represented by Child-Pugh score classes.

Outcome of the Patients

In our study it was found that out of 100 patients, 77 (77%) were discharged and 23 (23 %) patients expired.

It was also seen in our study that out of 25 patients who belonged to the group Ia – 16 (64%) patients expired and 9 (36%) patients were discharged.

Out of Ia group 64% patient expired and 36% discharged.

Out of Ib group, 90% patients were discharged and 9% patients expired.

Out of the group II, 90% patients were discharged and 9% patients expired.

The above data on application of Chi square test, p value < 0.0005 was obtained and was found to be statistically significant indicating that group Ia showed significantly grave outcome.

Serum and ascitic fluid TNF alpha and IL 6 levels of 100 patients were correlated with mortality and they were found to be statistically significant.

Similarly, Serum and ascitic fluid TNF alpha and IL 6 levels of group Ia were correlated with mortality and they were found to be statistically significant with a p value of 0.0005 on applying chi square test. These levels are also significant in terms of mortality in patients of group Ia.

The study also showed that Serum AST, ALT,

Bilirubin, Creatinine levels, and Child Pugh Score (LFT, KFT and severity of liver disease) significantly correlated with Serum and ascitic fluid TNF alpha and IL 6 levels.

Conclusion

Thus our study concludes that Serum and Ascitic fluid TNF alpha and IL 6 measurement on the day of admission of our patients as an easy and non-invasive method for both diagnosis and prognosis of our Chronic Liver Disease patients and thereby also paving way for the investigational role of Anti-TNF alpha and Anti -IL 6 molecules as a prospective cure for the patients.

The healthy liver has well developed defence mechanisms that permit hepatocytes to adapt to cytokine initiated stress, protecting them from cytokine-mediated lethality. TNF alpha may cause liver injury when hepatocytes have been pre-exposed to toxins such as alcohol, that interfere with their usual protective responses. Thus antagonism of TNF alpha and other injury-related cytokines in liver diseases merits evaluation as a possible treatment modality of these diseases.⁽⁵⁾

However, because the same cytokines are also necessary for the regeneration of tissue after liver injury, inhibition of these mediators might impair hepatic recovery.

We conclude that in cirrhotic patients, since the serum levels of both TNF alpha and IL-6 are higher in SABP than in sterile ascites, they are likely to play an important role in the pathogenesis of SABP. It has been suggested that TNF alpha production may enhance liver cell injury and that this will lead to renal impairment. This may account for the poor prognosis associated with SABP. We therefore recommend the measurement of serum TNF alpha and IL-6 in cirrhotic patients with SABP, as they have both diagnostic and prognostic significance.

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