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Original Research Article

Evaluation of Serum Interleukin-10 Levels in Cirrhotic Patients with Spontaneous Bacterial Peritonitis

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Abstract: Spontaneous bacterial peritonitis (SBP) is a very common bacterial infection in patients with cirrhosis and ascites. In these patients, cytokines as IL-10 are released to blood in response to infection. This study aimed to evaluate serumInterleukin-10 levels in cirrhotic patients with spontaneous bacterial peritonitis and its role in prediction of SBP. We prospectively studied 65 subjects at our University Hospital, The subjects were subdivided into three groups; group I included 20 healthy subjects (control group), group II included 20 cirrhotic patients without SBP and group III included 25 cirrhotic patients with SBP. IL-10 levels in the serum of studied groups were analyzed by ELISA and measured at diagnosis and after resolution for SBP group. A significant difference in serum IL-10 levels was detected between studied groups (P=0.0001) with mean value was higher in cirrhotic patients with SBP than cirrhotic patients without SBP and control group (67.51±34.03 vs 26.50±13.03, 17.08±5.20pg/mL respectively).Furthermore, There was significant decrease in the levels of serum IL-10 after treatment than before treatment (at diagnosis) in SBP group. Serum IL-10 at cut off value (19.4 pg/ml) had high sensitivity, specificity, PPV and NPV (80%, 80%, 90%, and 64% respectively) with AUC was (0.864) and (p=0.0001). IL-10 levels in serum may be related to development of SBP. Serum IL-10 had high sensitivity and specificity in prediction of SBP.
Keywords: Spontaneous bacterial peritonitis, Liver cirrhosis, Interleukin-10.

INTRODUCTION:

Spontaneous bacterial peritonitis is a bacterial infection of a previously sterile ascitic fluid developed in patients without any intra-abdominal, surgically treatable source of infection[1].The diagnosis of SBP was established by the presence of a polymorphonuclear cell count in ascitic fluid ≥ 250cells/mm^{3}in the absence of intra-abdominal source of infection [2]. SBP is one of the potential life-threatening complications in ascetic cirrhotic patients with a mortality rate ranging between 30-50% [3].

SBP can be caused by many reasons due to the alterations of the immune system that are very common in patients with end-stage liver disease and associated with an increased risk of infection and death [4, 5]. Consequently, elevated concentrations of proinflammatory cytokines are found in these patients. Moreover, anti-inflammatory cytokine are released to regulate this inflammatory reaction [6].

Interleukin (IL)-10 is a pleiotropic, anti-inflammatory, immunoregulatory cytokine that is important in protecting the host from infection-associated immunopathology, autoimmunity, and allergy. IL-10 was initially characterized as a T helper (T_H) 2 specific cytokine [7]; however, further investigations revealed that IL-10 production was also associated with T regulatory (Treg) cell responses [8-10].

The aim of the present study was to evaluate serum Interleukin-10 levels in cirrhotic patients with spontaneous bacterial peritonitis and its role in prediction of SBP.

PATIENTS AND METHODS:

This prospective study was conducted on 45 patients with post-hepatitis C liver cirrhosis and ascites attending to the Department of Hepatology, Gastroenterology and Infectious Diseases in Benha University Hospital from November 2015 to February 2016.In addition to 20 healthy subjects served as a control group, after approval of studied protocol by the

committee of ethics of scientific research of Benha Faculty of Medicine. The selected participants were subdivided into three groups; group I included 20 healthy subjects, group II included 20 patients without SBP and group III included 25 patients with SBP. They were selected for this study according to the following criteria:

Inclusion Criteria:

a. Age >18 years old.
b. Patients with post-hepatitis C liver cirrhosis and ascites.
c. Cirrhotic patients with SBP

Exclusion Criteria:

a. Patients with liver cirrhosis due to etiologies other than HCV infection as: (Co-infection with HBV).
b. Patients with any cause of ascites rather than liver cirrhosis e.g. hemorrhagic ascites, ascites from pancreatitis, tuberculosis or secondary to carcinomatosis.
c. Patients exposed to antibiotics within 2 weeks
d. Patients with secondary peritonitis.
e. Intra-abdominal infection e.g. Abscess, appendicitis, cholecystitis, and pancreatitis.

Written informed consent to participate in the study was obtained from all participants. After that, they were subjected to full history taking; clinical examination and routine laboratory investigation (CBC, liver function tests, kidney function tests, serum Na, serum K, and ESR).Serum IL-10 levels were assessed for all studied subjects at base line. Reassessment of serum IL-10 was done after appropriate antibiotic treatment for SBP patients (group III) by cefotaxime 4 g/day for 5 days [11] and ascitic fluid PMN count became < 250 cells/mm^3.

Estimation of serum IL-10(Pg/ml) by enzyme linked immunosorbent assay (ELISA):

A venous blood sample (2ml) was taken from each subject in studied groups. It was left to clot for half an hour and then centrifuged for 15 minutes at 1000 ×g. Serum was then aliquoted and stored at -80°C. Repeated freeze-thaw cycles were avoided. Hyperlipidemic and hemolysed samples were excluded. IL-10 concentrations were measured using a commercial ELISA kit (Shanghai Sunred Biological Technology, China).The assay range was 10pg/ml: 3000pg/ml and its sensitivity was 9.012pg/ml. The optical density of the developed color was measured at wave length 450nmusing TECAN Infinite F50 ELIZA Reader (Singapore) [12].

STATISTICAL ANALYSIS

Statistical presentation and analysis of the results was performed using SPSS.version 20.0 (Chicago, IL, USA) for data management.

STATISTICAL TESTS:

Quantitative data were expressed as mean ± standard deviation (SD) while qualitative data were presented as frequencies and percentage. Unpaired student t-test (two sided) was used to test the significance of difference between the mean value of two groups, ANOVA test was used to test the significance of difference between the mean value of more than two groups and chi-square test (χ^2) was used for comparison of categorical variables. Correlations between data were performed using Pearson correlation tests. ROC curve was used to determine cut off value of IL-10 with optimum sensitivity and specificity in prediction of SBP. Differences were considered significant when p value was < 0.05.

RESULTS:

The demographic data of the studied groups in our study revealed that the mean age of studied group was (49.53±14.98) years and the majority of cases were males [42 subjects (64.6%)] as shown in figure (1), rural [33 subjects (50.8%)], non-farmer [43 subjects (66.2%)], non-smoker [43 subjects (66.2%)], not diabetic [44 subjects (67.7%)] and not hypertensive [61 subjects (93.8%)].Table (1).

Table 1: Demographic features of the studied groups

Variable		**Mean ± SD or No (%)**
Age (years)Range:(22-80)	**(Mean ± SD)**	49.53±14.98
Sex, No. (%)	Male	42(64.6%)
	Female	23(35.4%)
Residence, No. (%)	Urban	32(49.2%)
	Rural	33(50.8%)
Occupation, No. (%)	Farmer	22(33.8%)
	Non-Farmer	43(66.2%)
Habit, No. (%)	Smoker	22(33.8%)
	Non-Smoker	43(66.2%)
D.M, No. (%)	No	44(67.7%)
	Yes	21(32.3%)
H.T.N, No. (%)	No	61(93.8%)
	Yes	4(6.2%)

Results are expressed as mean ± SD or frequency (%) as required.DM: Diabetes mellitus; HTN: Hypertension.

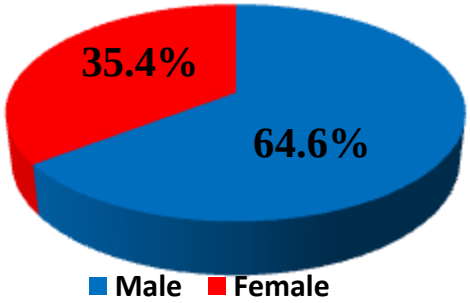

Fig 1: Sex distribution among studied groups.

The mean value and standard deviation in addition to minimum and maximum of all studied groups regarding blood picture, liver biochemical tests, renal function tests, electrolytes and ESR are presented in Table (2). Our result revealed that the majority of studied patients were Child C (71.1%) and Child B represents (28.9%) while no patients were Child A. Table (3). What is interesting in these result is that serum IL-10 levels were higher in SBP group than non-SBP and control groups with highly statistically significant difference (P=0.0001).Table (4) and there was decrease in levels of serum IL-10 after treatment in SBP group with highly statistically significant difference (P=0.007)as in Figure (2).

Table 2: Laboratory findings of the studied groups

Variable	Minimum	Maximum	Mean±SD
HB (g/dl)	3.90	15.50	10.84±2.76
WBC × 10^3 (cell/cmm)	2.80	24.80	8.17±4.37
PLT× 10^3 (cell/cmm)	25	384	163.26±103.86
AST (IU\L)	17	484	58.88±61.54
ALT (IU\L)	12	316	52.94±43.62
Serum albumin (gm /dl.)	1.90	5	3.18±0.76
Serum bilirubin(mg/dl)	0.20	18	3.70±4.30
PT (second)	11.60	27	15.02±2.90
INR	0.90	3.10	1.34±0.40
Serum creatinine (mg/dl)	0.50	6.20	1.39±0.89
Serum Na (mmol/L)	116	146	135.04±5.56
Serum K (mmol/L)	2.80	5.40	4.17±0.58
ESR (mm/hr.)	5	150	48.57±33.62

Table 3: Classification of the studied patients in group II (Non-SBP) and group III (SBP) according to the Child - Pugh classification

Child-Pugh Score	Number of Cases	%
Child (A)	0	0
Child (B)	13	28.9
Child (C)	32	71.1

Table 4: Comparison between studied groups regarding serum IL-10 levels

Variable	Group I (Control) N=20	Group II (Non- SBP) N=20	Group III (SBP) N=25	P value
Serum IL-10 (pg/ml) Mean ± SD	17.08±5.20	26.50[a]±13.03	67.51[ab]±34.03	**0.0001*** **

a: significant against group I
b: significant against group II

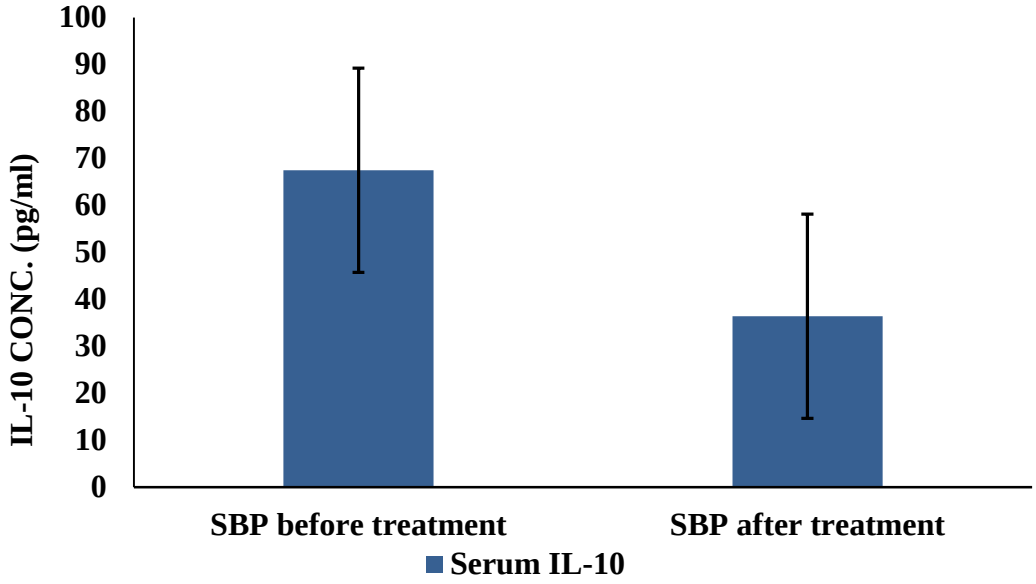

Fig 2: Serum IL-10 levels before and after treatment.

Table 5: Diagnostic performance of serum IL-10 in prediction of SBP

Variable	Cut off Value	Sensitivity	Specificity	PPV	NPV	AUC	Accuracy	P value
Serum IL-10	19.4 Pg/ml	80%	80%	90%	64%	0.864	80%	**0.0001*** **

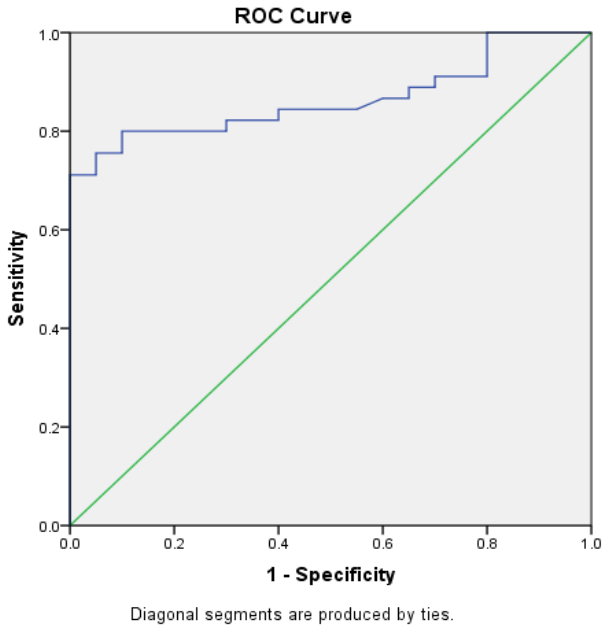

Fig 3: Sensitivity and specificity of serum IL10 in prediction of SBP

Diagnostic performance of serum IL-10 in prediction of SBP detected that serum IL-10 at cut off value (19.4 pg/ml) had high sensitivity, specificity, PPV and NPV (80%,80%,90%, 64% respectively) in prediction of SBP with AUC was (0.924) and (P = 0.0001). Table (5) and Figure (3).

DISCUSSION:

Spontaneous bacterial peritonitis is a serious complication of cirrhosis and has a high mortality rate unless diagnosed and treated early. Patients who develop SBP may have serum characteristics that are different from those who do not develop infection [13]. SBP is associated with a new increase in proinflammatory cytokines who could be antagonist by the concomitant elevation of several anti-inflammatory agents [6]. IL-10 is an anti-inflammatory immuno regulatory cytokine of Th2 immune response, regulating the extent of inflammation and the production of inflammatory cytokines [14].Our study assessed the levels of serum IL-10 in cirrhotic patients with and without SBP. And this study revealed a significant increase of serum IL-10 in SBP group than non SBP and control groups. This result is in agreement with previous studies [14-16] which revealed higher levels of serum IL-10 in SBP patients with statistically significant difference between SBP and non SBP groups**.** This can be explained as cytokines play an important role in host defense mechanism, and it is only under certain condition that may mediate deleterious results and contribute to the manifestations of tissue injury [17].

Interestingly, a significant decreases in the mean levels of serum IL-10 in SBP patients was detected after SBP treatment (p=0.007). These results support previous studies [6, 14, 15] into this brain area. This may be explained as the cytokines were consumed in large quantity in response to treatment when ascites are not well controlled [14]. These findings suggest that IL-10 plays a pathophysiological role during the development and the course of SBP.

This prospective study evaluated the diagnostic performance of IL-10 levels in serum of SBP patients and revealed that serum IL-10 at cut off value (19.4 pg/ml) had high sensitivity, specificity, PPV and NPV (80%, 80%, 90%, 64% respectively) with AUC was (0.864) and (p=0.0001) in prediction of SBP. But we did not find any available literatures discuss this point.

CONCLUSION:

Serum IL-10 is significantly increase in SBP group than other studied groups and significantly decreases after appropriate treatment of SBP. From this we can suggest that serum IL-10 may be related to the development of SBP and have a role in prediction of SBP.

CONFLICTING INTERESTS:

The authors declare that they have no conflicting interests.

AUTHORS' CONTRIBUTIONS

Elshawarbi GR participated in data collection from the patient records and collected blood for the study and performed the statistical analysis and drafted the manuscript. Omar MZ assisted with the study design, interpretation of results and discussion. Mohammed SA performed the laboratory analysis of the study, participated in the interpretation of results, designing and writing the manuscript. Nassar AK coordinated this work all through and revised the manuscript. All authors approved the final manuscript.

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