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Original Research Paper

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EFFECT OF INTRAVENOUS ALBUMIN ON INFLAMMATORY MARKERS IN INFECTIONS OTHER THAN SPONTANEOUS BACTERIAL PERITONITIS IN CIRRHOSIS

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ABSTRACT

Objective To study the effect of administration of intravenous albumin on inflammatory markers in Non-Spontaneous Bacterial Peritonitis (non-SBP) infections in patients with cirrhosis. **Materials &Methods** This study is a hospital based prospective randomized case control study of cirrhotic patients with non-SBP infections conducted over a period of two years. A total of fourty three cirrhotic patients with non-SBP infections s, subdivided into two subgroups, were included in the study. The patients in albumin group (n=20) received intravenous albumin at a dosage of 1.5 g/kg body weight at day one and 1.0g/kg body weight at day three whereas patients in control group (n=23) received supportive treatment only. Plasma levels of tumour necrosis factor (TNF)- , interleukin (IL)-6 and high sensitivity C-reactive protein (hs-CRP) were analysed before and after giving albumin. **Results** Patients in albumin group showed decrease in plasma levels of TNF- , IL-6, hs-CRP but only decrease in hs-CRP was statistically significant(p= 0.014) however patients in control group showed increase in TNF- ,IL-6, hs-CRP levels but increase in TNF- and IL-6 were statistically significant ($p \leq 0.0001$&$p \leq 0.0001$respectively). **Conclusion** The results show that the beneficial effects of albumin in non-SBP infections are related to the reduction in the plasma levels of TNF- , IL-6 and hs-CRP.

KEYWORDS : Albumin, Infection, Cirrhosis, Spontaneous bacterial peritonitis.

Introduction

Cirrhotic patients have a high risk of infection due to immunocompromised state. As many as 30-50% of deaths in cirrhotic patients are due to bacterial infections. Studies have shown that bacterial infections in cirrhotic patients are associated with poor outcomes (upto4-fold mortality). Patients who survive an episode of severe infection are still at high risk of death within a year [1].

Cirrhotic patients respond to infection with a greater increase in the circulating levels of cytokines like TNF- and IL-6 than patients without cirrhosis[2].TNF- is a macrophage derived cytokine produced in large amounts in response to endotoxin. Cirrhotic patients with SBP who develop kidney injury show higher plasma levels of TNF- than patients who maintain a stable kidney function. IL-6 is also a macrophage-derived cytokine that is partly induced by TNF- . Recent studies have showed a positive correlation between plasma IL-6 levels and the mortality rate of cirrhotic patients with SBP [3].

The exaggerated systemic response to infection in cirrhosis is due to bacterial translocation and release of bacterial products (endotoxins, proteases, lipopolysaccharides, peptidoglycans). These products stimulate the release of Nitric Oxide (NO) and inflammatory cytokines like TNF- , IL-1,IL-6.The effects of NO on the smooth muscle and endothelial cells results in refractory hypotension. This bacteria induced 'cytokine storm' leads to sepsis-related organ failures [4,5].

C-reactive protein (CRP), named for its capacity to precipitate C-polysaccharide of Streptococcus pneumoniae , is used as a marker of infection in different settings. It is synthesized in the acute phase of inflammation mainly in response to IL-6 [6].CRP has been evaluated both for the diagnosis of Systemic Inflammatory Response Syndrome (SIRS) and for prediction of short-term mortality in cirrhosis.[7]

The intravenous administration of albumin prevents the circulatory dysfunction and the development of kidney injury in patients with Spontaneous Bacterial Peritonitis (SBP) [8] .The beneficial effects of albumin in SBP are related to the decrease in the levels of TNF- and Nitric Oxide in both plasma and ascitic fluid [3].Albumin has a capacity to bind numerous substances including cytokines reducing the delivery of these substances to the cardiac myocytes and vascular endothelium [9].

Since the release of cytokines and vasodilators is a well-known feature of all bacterial infections and not only of SBP, it may be inferred that albumin may be acting in a similar way in non-SBP infections. Therefore we undertook this prospective study to know the effect of albumin on inflammatory markers in infections other than SBP in cirrhotic patients.

Materials and Methods

Patient Selection

It was a hospital based prospective randomized case control study conducted over a period of 2 years in the Depatment of Gastroenterology Sher-i-Kashmir Institute Of Medical Sciences (SKIMS),Soura Srinagar after proper clearance from the institutional ethical committee.

Fourty three patients with diagnosis of cirrhosis who fulfilled the eligibility criteria were included in the study.

Inclusion criteria :

(1)Cirrhosis defined by clinical, lab or radiological findings
(2)Presence of an infection on the basis of radiological findings and complementary methods or suspicion of infection based on at least one of the following: blood polymorphonuclear cell (PMN) count >10,000/mm^3 or ≥50% increase with respect to baseline with a final value >8,000/mm^3; more than 5% of band forms; and/or temperature

>37.5^{0}C or when 2 or more of the SIRS criteria are present .
(3) Serum creatinine<3mg/dl.

Exclusion criteria :
(1)Presence of SBP
(2)Difficult to treat infections such as endocarditis, septic arthritis ,osteomyelitis . (3)Heartfailure(stage3-4)
(4)Gastrointestinal bleed during preceding week .
(5)Recent antibiotic treatment during preceding week(except norfloxacin prophylaxis).
(6) Septic shock
(7)Hepatocellular carcinoma-Stage D
(8)Disease which can influence short time survival(Human immunodeficiency virus infection, advanced neoplasm).

Protocol
A complete history, physical examination, routine laboratory tests, and cultures of blood, urine ,and ascetic fluid or other organic fluids,if indicated, was performed in all patients before inclusion in the study.

Randomization was done within the first 24h after diagnosis of the infection. The patients in albumin group received intravenous albumin at a dosage of 1.5g/kg body weight at day one followed by 1.0g/kg body weight at day three in addition to supportive treatment whereas patients in control group received supportive treatment only. All the patients were managed with the standard supportive treatment which was similar in both the groups. Plasma concentrations of TNF- , IL-6 and hs-CRP were analysed in both the groups. These inflammatory markers were done on day 0 (before giving albumin) and day 6 (after giving albumin).

The patients received treatment and prevention for the complications. The empiric antibiotic treatment was based on therapeutic protocols used in our hospital at the time of the study design. The initial antibiotic treatment was modified according to the culture and sensitivity reports. Antibiotics were continued until the disappearance of signs and symptoms of the infection, normalization of the white blood cell count.

Laboratory measurements
Peripheral blood (4ml) was collected from patients by venipuncture following universal precautions and under all asceptic conditions in sterile collection tubes. The tubes were subjected to centrifugation and stored at-80°C till assayed for TNF- , IL-6 & hs-CRP levels.

Quantitative measurement of inflammatory markers
Enzyme linked immunosorbent assays (ELISAs) for the quantitative measurement of TNF- , IL-6 and hs-CRP levels were used.

TNF- and IL-6 were estimated by using commercially available ELISA Kit from DIACLONE, FRANCE. hs-CRP was estimated by using ELISA Kit from DIAGNOSTICS BIOCHEMCANADAINC. All the assays were carried out following the manufacturers protocol. The absorbance was recorded at 450 nm using an automated ELISA reader which recorded the absorbance and calculated the corresponding concentration of the marker.

A linear standard curve was generated by plotting the average absorbance of each standard on the vertical axis versus the corresponding marker (TNF- , IL-6 or hs-CRP) standard concentration on horizontal axis. The amount of marker in each sample was determined using the standard curve.

Definitions
Renal failure was diagnosed in patients who had serum creatinine greater than1.5mg/dl at inclusion in the study or whenever serum creatinine increased by more than 50% with respect to baseline value with a final value over 1.5mg/dl during the admission period.

SIRS was defined as two or more of the following: Temperature>38°Cor <36°C; heart rate > 90 beats per minute; respiratory rate > 20 breaths per minute or PaCO2<32mmHg ; White cell count > 12,000/mm^3or< 4,000 mm^3, or the presence of > 10% immature neutrophils .

Statistical Analysis.
Patients were analysed in the two groups. Frequency distribution was assessed in terms of mean ± SE (standard error) for quantitative variables and numbers (percentages) for categorical variables. Statistical analysis was carried out using the Mann-Whitney U-test and the non-parametric paired Wilcoxon test for quantitative variables, and the (2 test for qualitative variables, applying the Fisher exact test when required. Results were expressed as mean value (SE). P value(<0.05)was considered statistically significant.

Results
At enrollment both the groups were comparable in majority of clinical and biochemical parameters.Table1 shows the baseline characteristics of both the groups. Majority of patients in both the groups were males. The most common presentation to hospital was encephalopathy in both the groups.

Table1.Baseline characteristics of albumin and control groups

CHARACTERISTIC	ALBUMIN GROUP	CONTROL GROUP
Number of patients	20	23
Age(years)	53(12)	55(13)
Male/Female	12/8	14/9
Child Pugh score	9.5(3.2)	9.8(2.3)
ETIOLOGY OF CIRRHOSIS-NO.(%)		
Chronic Hepatitis B	12(60)	15(65)
Chronic Hepatitis C	6(30)	7(30)
Alcoholism	2(10)	1(5)
Serum albumin(g/dl)	2.5(0.5	2.6(0.6
Serum bilirubin(mg/dl)	4.2(3.2	4.4(4.1
Serum creatinine(mg/dl)	1.2(0.9	1.2(0.7
Serum sodium(mEq/l)	126(6	129(5
TYPE OF INFECTION NO(%)		
Pneumonia	6(30)	7(30)
Urinary tract	8(40)	10(44)
Skin	2(10)	1(5)
Occult	4(20	5(21)

Continuous variables are expressed as mean values (SE. Data are presented as number and percentage of total.

Ten (23%) of the 43 patients in both the groups had infection confirmed with positive cultures. In the remaining patients, infection was suspected because of fever and/or increased leukocyte count. Gram negative organisms were the major pathogens isolated ,found in eight patients and gram positive organisms in two patients (Table2).

Table2.Pathogens isolated in both the groups.

Organism	Albumingroup n=20	Controlgroup n=23
Escherichia coli	2	3
Klebsiella pneumoniae	1	1
MRSA	1	1
Acinetobacter baumannii	1	0

Abbreviations: MRSA=Methicillin resistant staphylococcus aureus

The clinical response to treatment and survival was similar between albumin and control group (Table3). Improvement in hepatic encephalopathy (H.E) was seen in 18 patients in albumin group and 19 patients in control group. One patient each from the two groups had in hospital GI bleed. During the whole hospitalization period 2 patients from albumin group (10%) and 4 patients from control group(17%) died. 3 patients from albumin group (15%) and 6 patients from control group(26%) had renal failure during the study period. There was no statistical significance between the two groups in the incidence of renal failure and in-hospital mortality. The causes of in-hospital mortality were renal failure (1 patient in albumin group and 2 patients in control group), gastrointestinal bleed (1 patient in each group), pulmonary edema (1 patient in control group)

Table3.Clinical response and outcome in relation to markers

Albumin group Parameters-No.(%)		Control group	pvalue
Number of patients	20	23	
Encephalopathy improvement	18(90)	19(82.6)	0.66
Gastrointestinal bleed	1(5)	1(4.3)	1.00
In-hospital mortality	2(10)	4(17)	0.66
Renal failure	3(15)	6(26)	0.46

Data are presented as number and percentage of total. *p(0.05.

After antibiotic plus Albumin treatment, patients in Albumin group showed decrease in plasma levels of TNF- , IL-6 and hs-CRP (Table4), but only decrease in hs-CRP was statistically significant (p value=0.014). After treatment with antibiotics only, patients in Control group showed increase in plasma levels of TNF- , IL-6 and hs-CRP

(Table5) but increase in plasma levels of TNF- and IL-6 were statistically significant (pvalue ≤0.0001 and pvalue ≤0.0001, respectively).

Table4.Changes in plasma levels of inflammatory markers in Albumin Group

Markers	Mean±S.E	Pvalue
TNF-αD0	41.4±10.5	0.433
TNF-αD6	30.5±8.8	
IL-6D0	166.1±27.1	0.126
IL-6D6	136.2±26.8	
hs-CRPD0	12614±606	0.014
hs-CRPD6	10923±967	

Table5.Changes in plasma levels of inflammatory markers in Control Group

Markers	Mean±S.E	pvalue
TNF- D0	51.6±17.4	(0.0001
TNF- D6	209.6±90.4	
IL-6D0	128.3±18.9	(0.0001
IL-6D6	246.4±13.4	
hs-CRPD0	11707±948	0.738
hs-CRPD6	122021±716	

Abbreviations: TNF- =tumor necrosis factor alpha; IL=interleukin; hs-CRP=high sensitivity C-reactive protein. Values of TNF- and IL-6 are in pg/ml; hs CRP in ng/ml. Values are expressed as means (SE.
*p (0.05.

Discussion

This study is consistent with several findings of previous studies. In previous studies related to infections in cirrhotics , urinary tract infection (UTI) has been the most common non-SBP infection observed followed by pneumonia & skin infection [8,13].Distribution of infection in our cohort also showed urinary tract infection (UTI) as the most common infection seen followed by pneumonia, occult & skin infection in cirrhotic patients.

TNF- α and IL-6 are macrophage derived cytokines which are markedly increased in cirrhotics with bacterial infections more so in patients developing renal dysfunction [3].A similar feature in our study reveals that TNF- andIL-6 are markedly increased in non-SBP infections in cirrhotic patients. The exaggerated systemic response to infection in cirrhosis is due to bacterial translocation and subsequent release of bacterial products importantly endotoxin of gram negative bacteria .The endotoxin stimulates the release of inflammatory cytokines and nitric oxide which act on endothelial and smooth cells resulting in multiple organ dysfunction.[5] In addition cirrhotic patients have reduced ventricular contractibility and bacterial infections produces a cytokine mediated septic cardiomyopathy with TNF , playing a major role. [9] Renal dysfunction is present in one third of SBP cases and one of the main causes of in-hospital death in these patients . Since the release of cytokines is a common feature of all bacterial infections , renal failure in cirrhotics is also induced by non-SBP infections [14]. Cirrhotic patients with renal failure due to infection other than SBP have a poor prognosis with in hospital mortality of 42.8% (7.24% in cases without renal failure)[13].

The most striking finding in this study is the decrease in plasma levels of inflammatory markers following antibiotic plus albumin treatment and the significant increase in the marker levels without albumin infusion. The high plasma levels of TNF- , IL-6 and hs-CRP decreased, in fact hs-CRP levels decreased significantly after treatment with antibiotics plus albumin in this study.

Human serum albumin, the most abundant plasma protein is used as an intravascular volume expander. The main physiologic function is to maintain colloid osmotic pressure. Albumin is the recommended treatment for prevention of acute renal failure in patients with SBP[15].

The beneficial effect of albumin has been related to its volume expansion, antioxidant effects, capacity to bind cytokines reducing the delivery of these substances to cardiac myocytes and vascular endothelium.[9]

The beneficial role of albumin in non-SBP infections in cirrhotic patients has been demonstrated previously in two studies. Monica G et al in their prospective randomized study have shown that in non-SBP infections, administration of albumin in addition to an antibiotic improves renal & circulatory function significantly, reduces frequency of type1 hepatorenal syndrome & shows a potential survival benefit as compared with standard antibiotic therapy alone. There was a significant decrease in plasma renin activity, plasma aldosterone and norepinephrine concentration and increase in plasma atrial natriuretic peptide levels following albumin infusion in their study.[8]Thierry Thevenot et al revealed in their study that albumin infusion delayed the onset of renal failure but did not improve renal function or survival at 3 months [17].These results support the use of albumin in infections other

than SBP in cirrhotic patients. However intravenous albumin is expensive and has limited availability in some settings. Besides potential infection introduced via albumin administration, it is a drug with some adverse effects which occur at a very low rate which include allergic reaction in a patient who has known allergy to albumin and fluid overload particularly in decompensated Congestive heart failure and severe anemia patients [16].In the current study two patients developed signs of pulmonary edema which was attributed to albumin administration.

In summary, this study shows that non-SBP infections like SBP are associated with activation of cytokine cascade. It is the first prospective study to evaluate the beneficial effects of intravenous albumin infusion on inflammatory markers in infections other than SBP in cirrhotics .To derive, the beneficial effects of albumin in non-SBP are related to the reduction of the levels of Inflammatory cytokines. We suggest that further studies with large sample size should be undertaken to confirm these findings.

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