Research Article

"IL17 expression in spontaneous bacterial peritonitis in HCV related liver cirrhosis and its correlation with the development of acute kidney injury."

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Abstract
Introduction: SBP is a potentially lethal complication of ascites. IL-17A is a proinflammatory cytokine released by T cells that have a role in host immune defense and inflammation. Intestinal Paneth cells have been shown to synthesize IL-17A and respond to inflammatory stimuli by overproducing IL-17A. This mechanism has recently been implicated in liver-related acute kidney injury. **Aim of the work:** Study the diagnostic performance of IL17A in the ascetic fluid as a marker of ascitic fluid infection and whether patients with elevated IL17A in the setting of SBP are at a higher risk for the development of AKI. **Patients and Methods:** This cross-sectional prospective, case-control hospital based study included 60 cirrhotic patients; 40 patients with cirrhotic ascites secondary to HCV but not with SBP and 20 patients with cirrhotic ascites secondary to HCV with SBP. **Results:** we found a strong relationship between levels of serum and ascitic IL 17 and acute deterioration of liver and renal function.
Keywords: ALB: Albumin, AP: Alkaline phosphatase, ALT: Aminotransferases Alanine

Introduction
SBP is a potentially lethal complication of ascites. SBP is an infection of ascites that occurs in the absence of a contiguous source of infection (e.g., intestinal perforation, intraabdominal abscess) and in the absence of an intraabdominal inflammatory focus (e.g., abscess, acute pancreatitis, and cholecystitis). Since its first description in the English literature in 1963-1964 as a "rarely recognized syndrome," great strides have been made in its recognition and therapy, leading to a decrease in its mortality from 80% or more in the initial series to a current mortality rate of 10% to 20% (Garcia-Tsao, 2004).

A diagnostic paracentesis is the mainstay in the diagnosis of SBP and should be performed not only in cirrhotic patients with ascites who have compatible clinical features (e.g., fever, abdominal pain, leukocytosis) but also in those admitted to the hospital for any other reason and in those with unexplained encephalopathy or renal dysfunction (Runyon, 2009; Rimola et al., 2001).

The diagnosis of SBP is established with an ascites PMN count greater than 250 per mm3. Because SBP is associated with a high mortality without timely treatment, there should always be a high index of suspicion for it. SBP is a frequent cause of hepatic encephalopathy, HRS, and occult infection (Perumalswami and Schiano, 2011).

IL-17A is a proinflammatory cytokine released by T cells that have a role in host immune defense and inflammation. Intestinal Paneth cells have been shown to synthesize IL-17A and respond to inflammatory stimuli by overproducing IL-17A. This mechanism has recently been implicated in liver-related acute kidney injury (Park et al., 2011).

Takahashi and colleagues, using a mouse model of hepatic ischemia/reperfusion injury (which also develops significant renal dysfunction), demonstrated increased intestinal Paneth cell degranulation and increased IL-17A levels in the portal vein and small intestine (Takahashi et al., 2008).

Both an IL- 17A–neutralizing antibody and genetic deletion of IL-17A were found to be protective against hepatic ischemia/ reperfusion and kidney injuries. In addition, depletion of Paneth cells attenuated not only hepatic ischemia/reperfusion injury but also renal dysfunction in this model (Park et al., 2011), suggesting that Paneth cell–derived IL-17A may also have a role in the pathophysiology of renal dysfunction in cirrhotic patients.

Aim of the work

Study the diagnostic performance of IL17A in the ascetic fluid as a marker of ascitic fluid infection and whether patients with elevated IL17A in the setting of SBP are at a higher risk for the development of AKI.

Patients and Methods

This cross-sectional prospective, case-control hospital-based study was conducted in Internal Medicine Department, Minia University Hospital from June 2016 to June 2017. This study included 60 subjects who were recruited from inpatient and out-patient clinic and divided in to 2 groups:

GROUP (1): 40 patients with cirrhotic ascites secondary to HCV but not with SBP.

GROUP (2): 20 patients with cirrhotic ascites secondary to HCV with SBP are recruited from Minia University Hospital. SBP was diagnosed on the basis of a polymorphonuclear (PMN) cell count in ascitic fluid > 250 cells/mm3 in the absence of clinical, radiological, or laboratory data suggesting secondary peritonitis or other abdominal disorders resembling SBP (e.g., hemorrhage into ascites, pancreatitis, peritoneal tuberculosis, or carcinomatosis). Because many cases of SBP are culture-negative, the isolation of the responsible organisms was not considered essential for the diagnosis. Patients with bacterascites defined as the detection of bacteria in the presence of a normal ascites PMN count were not included in the study.

Diagnosis of chronic HCV infection was based on positivity of anti-HCV antibodies by ELISA and confirmed by detection HCV RNA by PCR (Wilkins et al., 2010).

Diagnosis of liver cirrhosis was based on the presence of clinical signs of portal hypertension (esophageal varices, splenomegaly and ascites), appropriate findings in abdominal ultrasound and standard laboratory data (Iacobellis et al., 2005).

Exclusion criteria: subjects had any one of the following criteria were excluded from study: Patient who have undergone abdominal surgery within the last month. Patient with chylous ascites or ascites not related to portal hypertension (pancreatic ascites, hemoperitoneum, ascites observed during acute heart failure, peritoneal tuberculosis, hepatocellular carcinoma).

All patients and control were subjected to the following: -

I) Thorough History Taking:

Subject answered a standard questionnaire that included:

Personal history with special attention to name, age, sex, weight, height, residence, marital status, occupation and any special habits of medical importance. Current history of symptoms suggestive of hepatic decompensation as fatigue, fever, bleeding tendency, itching, yellowish discoloration of sclera, change of color of urine and stools, abdominal distension, swelling of lower limbs, hematemesis, melena, Child-Pugh stage and disturbed level of consciousness.

II) Thorough Clinical Examination:

Measurement of vital signs (body temperature, pulse, blood pressure and respiratory rate). General examination with special emphasis on stigmata of chronic liver disease as jaundice, fetor hepaticus, palmar erythema, clubbing of fingers, flappy tremors, spider nevi, scratching marks, evidence of subcutaneous hemorrhage, gynecomastia, lower limb edema, and conscious level. Careful abdominal examination with special reference to the status of the liver and spleen as regards size, surface, edge, consistency and tenderness on examination, the presence of ascites was evaluated, abdominal tenderness or fever.

(III) Laboratory Investigations:

Blood Sampling Protocol: After 8-hour overnight fasting blood sample was drawn via sterile venipuncture at 8 A.M. Samples were divided into three blood collecting tubes: first tube containing ethylene diamine tetra acetic acid (EDTA) was used for complete blood count, the second one containing tri sodium citrate was used for determination of prothrombin time and concentration and lastly a plane tube. The plane tube was left to clot then centrifuged; separated serum was put into aliquot tube used for routine biochemistry tests including liver function test, renal function, viral markers. Renal function tests were repeated daily. IL17, liver function tests, white blood cell count, and ascitic fluid PMN cell count were repeated a week after diagnosis of SBP or 24 hours after antibiotic withdrawal (which is closer).

Results

We found that serum and ascitic levels of IL-17 in cirrhotic patients with spontaneous bacterial peritonitis were significantly higher than in patients with cirrhosis without spontaneous bacterial peritonitis. Also, we found significant decline in both serum and ascitic fluid IL17 levels with successful treatment of spontaneous bacterial peritonitis.

Also, it was found that these elevated levels of IL17 in serum and ascitic fluid were significantly associated with decline of baseline GFR and its improvement with successful treatment and reduction of IL 17 levels in serum and ascites, denoting the involvement of IL17 in extrahepatic – kidney-organ injury. Other finding included higher Child-Turcotte-Pugh, MELD and MELD-Na scores and lower ascitic fluid total protein among cases with SBP.

Table 1: serum and ascites IL 17 before and after treatment in patients with SBP.

	Mean ± SD	***p*-value**
Serum IL17	612.3±213	<0.001*
Follow up Serum IL17	182.5±126.8	
Ascitic IL17	648±197.4	<0.001*
Follow up Ascitic IL17	178.6±114.5	

Paired sample (t) test for analysis of quantitative variables
For all tests probability (p) was considered Significant if < 0.05

Table 2: Association of serum and ascites IL17 with age and severity of liver cirrhosis and ascites total protein levels in patients with SBP after treatment.

	Serum IL17		**Ascites IL17**	
	r	**p-value**	**r**	**p-value**
Age	0.111	0.641	0.111	0.642
Ascites total protein	-0.342	0.14	-0.286	0.222
Serum total Bilirubin	0.18	0.449	-0.072	0.763
Serum direct Bilirubin	0.129	0.588	0.007	0.978
Serum creatinine	0.173	0.466	-0.044	0.855
INR	0.213	0.368	0.05	0.835
MELD	0.24	0.308	0.071	0.765
MELD-Na	0.848**	≤0.001**	0.707**	≤0.001**

Serum and ascites IL17 are potential diagnostic tests for SBP

Variable	AUC	Sensitivity	Specificity	Cut off point	p-value
Serum IL17	1.000	100%	100%	>92	*<0.001**
Ascitic IL17	1.000	100%	100%	>132	*<0.001**

Discussion

We found that serum and ascitic levels of IL-17 in cirrhotic patients with spontaneous bacterial peritonitis were significantly higher than in patients with cirrhosis without spontaneous bacterial peritonitis; this is in agreement with (park et al., 2011) who developed a model for hepatic Ischemia-reperfusion (IR) injury and found that hepatic IR induced small intestinal Paneth cell degranulation and increased interleukin- 17A (IL-17A) levels (park et al., 2011).

They hypothesized that small intestinal Paneth cell derived IL-17A plays a critical role in generating liver, kidney, and intestine injury after hepatic IR. Their results support this hypothesis, as small intestinal Paneth cells degranulate and increase IL-17A production after liver IR, plasma and tissue levels of IL- 17A increase significantly with the highest IL-17A levels detected in portal vein plasma and in the small intestine, depletion of IL-17A with neutralizing antibody or genetic deletion of either IL-17A or the IL-17A receptor protected against liver IR injury and extrahepatic organ dysfunction, pharmacological (with dithizone treatment) or genetic depletion (with intestine specific SOX9 deletion) of Paneth cells attenuated hepatic, renal, and intestinal injury following hepatic IR, depletion of Paneth cell granules markedly decreased small intestinal IL-17A release and significantly attenuated plasma and tissue IL-17A levels after hepatic IR (Park et al., 2011).

Also, they Proposed that hepatic IR up-regulates small intestinal Paneth cell IL-17A production and Paneth cell derived IL-17A plays an important role in propagating multiorgan injury after hepatic IR. They demonstrated that rapid degranulation of small intestinal Paneth cells with induction of IL-17A after liver IR (Park et al., 2011). Small intestinal Paneth cells are crucial for both mucosal as well as innate immunity against pathogens and can actively secrete several antimicrobial peptides (e.g., lyso-zyme, α-defensins/cryptdins) as well as proinflammatory molecules (e.g., inducible NO synthase, phospholipase A2, IL-17A) (Bultinck et al., 2006; Huttner et al., 1994; Seno et al., 2002; Takahashi et al., 2008).

Conclusion and future recommendations

We found a strong relationship between levels of serum and ascitic IL 17 and acute deterioration of liver and renal function plus significant decline in both serum and ascitic fluid IL17 levels with successful treatment of spontaneous bacterial peritonitis. Also, we found association between SPB and lower ascitic fluid total protein and higher Child-Turcotte-Pugh, MELD and MELD-Na scores.

These results may provide additional method to diagnose patients with SBP, especially culture negative and non-neutrocytic SBP and Also, Measuring IL-17 level can provide a prognostic utility for severity of SBP and associated liver and kidney injury. Also, more focused studies regarding cut-off levels, sensitivity and specificity for diagnosing SBP should be carried out.

Future inclusion of spontaneous bacterial peritonitis in trials for treatment through modulation of IL17. Also, this may help protection from acute liver and kidney injury associated with SBP.

References

1. Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. Gastroenterology. 2001; 120(3):726–48.
2. Novella M, Solà R, Soriano G, et al., Continuous versus inpatient prophylaxis of the first episode of spontaneous bacterial peritonitis with norfloxacin. Hepatology 1997;25:532-6.
3. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al., A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6(11):1133-41.
4. Pascal J-P, Cales P, Multicenter Study Group. Propranolol in the prevention of first upper gastrointestinal tract hemorrhage in patients with cirrhosis of the liver and esophageal varices. N Engl J Med 1987; 317: 856-61.
5. Puel A, Cypowyj S, Bustamante J, Wright JF, Liu L, Lim HK, et al., Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science. 2011; 332(6025):65-8.
6. Puel A, Doffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, et al., Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. J Exp Med. 2010; 207(2):291-7.
7. Rabeony H, Petit-Paris I, Garnier J, Barrault C, Pedretti N, Guilloteau K, et al., Inhibition of keratinocyte differentiation by the synergistic effect of IL-17A, IL-22, IL-1alpha, TNFalpha and oncostatin M. PLoS One. 2014; 9(7): e101937.
8. Richards MH, Nelson JL. The evolution of vertebrate antigen receptors: a phylogenetic approach. Mol Biol Evol. 2000; 17(1):146-55.
9. Rimola A et al., Oral, nonabsorbable antibiotics prevent infection in cirrhotics with gastrointestinal hemorrhage. Hepatology. 2001;5(3):463–7.
10. Rolachon A et al., Ciprofloxacin and long-term prevention of spontaneous bacterial peritonitis: results of a prospective controlled trial. Hepatology. 1995; 22(4):1171–4.
11. Runyon BA. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. Hepatology 2009; 57: 1651-3.
12. Sort P et al., Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. N Engl J Med. 1999;341(6):403–9.
13. Wong, R.J., Gish, R.G. Metabolic Manifestations and Complications Associated With Chronic Hepatitis C Virus Infection. Gastroenterol. Hepatol. 2016; 12, 293–299.
14. Woolf GM, Runyon BA. Spontaneous Salmonella infection of high-protein noncirrhotic ascites. J Clin Gastroenterol. 1990; 12(4):430–2.
15. Zhang Y, Wang H, Ren J, Tang X, Jing Y, Xing D, et al., IL-17A synergizes with IFN-gamma to upregulate iNOS and NO production and inhibit chlamydial growth. PLoS One. 2012; 7(6):e39214.
16. Zrioual S, Ecochard R, Tournadre A, Lenief V, Cazalis MA, Miossec P. Genome-wide comparison between IL-17A- and IL-17F-induced effects in human rheumatoid arthritis synoviocytes. J Immunol. 2009; 182(5):3112-20.