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Calprotectin Measurement in Ascitic Fluid: A New Test for the Rapid Diagnosis of Spontaneous Bacterial Peritonitis

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Abstract

Introduction: Spontaneous bacterial peritonitis (SBP) is diagnosed by the presence of ❑250 polymorph nuclear cells (PMN) per milliliter (ml) in ascitic fluid. However, the procedure is time-consuming, operator-dependent, and not always readily available. Calprotectin is a cytosolic protein in neutrophilic leukocytes that is released in response to inflammatory processes. In the GI tract calprotectin in feces is considered a valid marker of intestinal inflammation.

Aims and Methods: The study aimed to assess the role of calprotectin concentrations in ascites for the diagnosis of SBP. Forty patients [mean age 50±2 years, 10 (25%) females] with HCV liver cirrhosis. Who were referred for paracentesis were included in this study; the following investigations were performed: Serum-ascites albumin gradient, ascitic PMN cell count (microscopic hand-count), bacterial cultures and cytological analysis. In addition, ascitic calprotectin was measured using an enzyme-linked immunosorbent assay (ELISA).

Results: Ascitic calprotectin values measured by ELISA correlated well with PMN cell count (p-value=0.001, rho—F0.729). Calprotectin levels of patients with PMN ❑250/m1 (N=8, 20%) were higher than in patients with PMN <250/ml (N=32, 80%). Ascitic calprotectin values correlated well with albumin level (p-value=0.001, rho=-0.716).

Conclusion: Measurement of calprotectin in ascites might be a valuable surrogate marker for PMN cell count. It might be valuable for the rapid diagnosis of SBP.

Key Words: *Calprotectin — Ascites — Spontaneous bacterial peritonitis.*

Introduction

SBP is the infection of the ascitic fluid that occurs in the absence of a visceral perforation and in the absence of an intra-abdominal inflammatory focus such as abscess, acute pancreatitis or cholecystitis. For SBP diagnosis, the number of polymorphonuclear leucocytes (PMN) from the ascitic fluid obtained by paracentesis must exceed 250 cells/mm^3 and from bacteriological cultures only one germ must be isolated (5-7). Because SBP is in most cases a monomicrobial infection, the presence of more microorganisms in the culture (>1), must raise the suspicion of secondary peritonitis [1].

Calprotectin is a 36-kDa calcium and zinc binding protein and constitutes approximately 60% of soluble cytosol proteins in human neutrophil granulocytes [2]. It comprises two heavy chains of 14 kDa and one light chain of 8 kDa. This protein has also been called MRP8, MRP14,2 cystic fibrosis-associated antigen [3] calgranulin [4] and S100 [5]. Finally, its name was changed to reflect its calcium binding and protective properties [6].

The name calprotectin has not met with uniform agreement as it actually refers to a heteromeric complex of two subunits, S100A8 and S100A9. It has been suggested that these may be differentially regulated in disease states *[7]* but free subunits are not found in biological samples unless the protein is heated in the presence of detergents to dissociate the non-covalent bonds between them. Calprotectin is a multipotent biologically active molecule [8]. It has been suggested that calprotectin plays an important role in myeloid cell metabolism [9]. When externalized to cells, calprotectin has immunomodulatory [10] and antimicrobial effects 1111.

Ascitic fluid calprotectin levels are significantly elevated in malignant ascites and spontaneous bacterial peritonitis, compared with ascites secondary to alcoholic liver disease and viral hepatitis. High ascitic calprotectin levels in cirrhotic patients appear to be predictive of high mortality [12].

Aim of the study: To evaluate the possible role of calprotectin concentrations in ascitic fluid for the diagnosis of SBP.

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Material and Methods

This study was conducted at the Tropical Medicine and Gastroenterology Department, Qena Faculty of Medicine from February 2012 — May 2012. Forty patients with hepatitis C related cirrhosis with ascites admitted to the department were recruited. Exclusion criteria were all causes of ascites other than cirrhotic ascites.

All patients were subjected to:

- Thorough history taking.
- Complete physical examination.
- Laboratory investigations including: (AST, ALT, bilirubin, serum albumin, prothrombin time and concentration and complete blood count).
- Ascitic fluid study was done for diagnosis of SBP and determination of ascitic albumin level.
- Ascitic calprotectin were detected by simple ELISA technique using polyclonal antibodies for capture and affinity purified. The calprotectin test was supplied by BioActiva Diagnostica GmbH, Germany.
- Abdominal ultrasonography: For assessment of liver and spleen size, portal and splenic vein diameter and the degree of ascites.

Results

This study included 40 patients with liver cirrhosis and ascites with their mean age 50±2 years. Eight (20%) of them showed SBP and 32 (80%) patients without SBP. Demographic and laboratory data for patients are illustrated in Table (1).

Table (1): Demographic and laboratory data for patients.

Patients (n=40)	Value
Age (yr)	50±2
Gender:	
Male, n (%)	30 (75%)
Female, n (%)	10 (25%)
SBP:	
Yes, n (%)	8 (20%)
Bilirubin (mg/dl)	2.87±1.102
Albumin (g/dl)	2.29±0.55
ALT (U/L)	47.12±30.50
AST (U/L)	57.80±50.88
Prothrombin time (sec)	17.108±3.45
Prothrombin conc. (%)	56.120±11.7

Data expressed as mean±SD

In this current study ascitic fluid study showed that PMN cell count was significantly higher in patients with SBP than those without SBP (p-value =0.000), also ascitic calprotectin level showed statistically significant higher value in patients with SBP than those without SBP (p-value=0.000). This is illustrated in Fig. (1).

Ascitic fluid culture showed that 85% were infected with G —ve organism (E coli) and 15% of the cultures were infected with G +ve (streptococci). Mean serum ascitic albumin gradient level was 2 .1±0.7 g/dl.

Positive correlation was detected between ascitic calprotectin and ascitic PMN cell count *(p-value=0.001,* rho=+0.729). This is illustrated in Fig. (2). Also positive correlation was detected between ascitic calprotectin and total serum bilirubin (p-value=0.000, rho=+0.661), Fig. (3).

On the otherhand, negative correlations were detected between ascitic calprotectin and serum albumin (p-value <0.001, rho=-0.716) and between ascitic calprotectin and prothrombin conc. (p-value =0.003, rho=-0.451), Figs. (4,5).

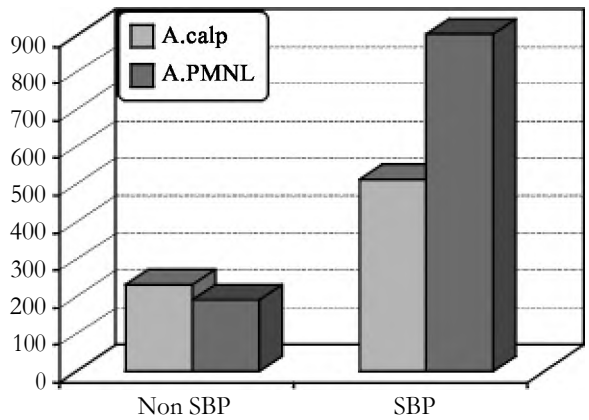

Fig. (1): Ascitic calprotectin and PMN cell count in patients with and without SBP.

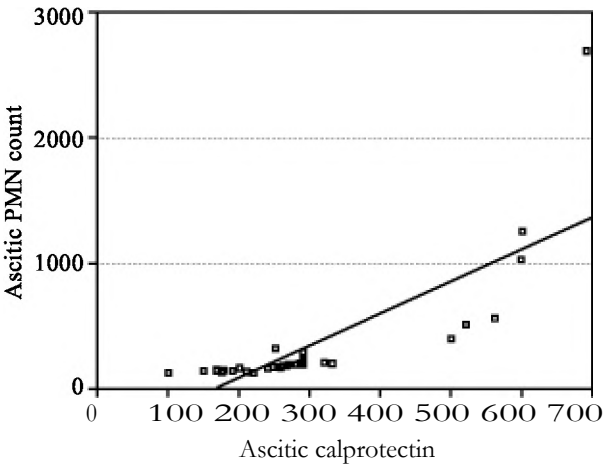

Fig. (2): Correlation between ascitic calprotectin and PMN cell count (p-value=0.001, rho—W.729).

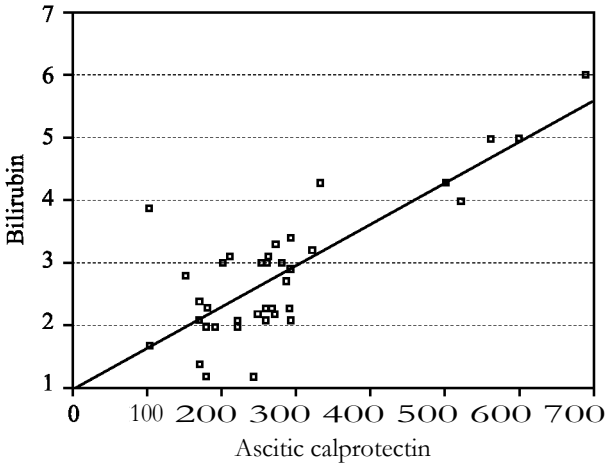

Fig. (3): Correlation between ascitic calprotectin and serum bilirubin (p-value=0.000, rho=+0.661).

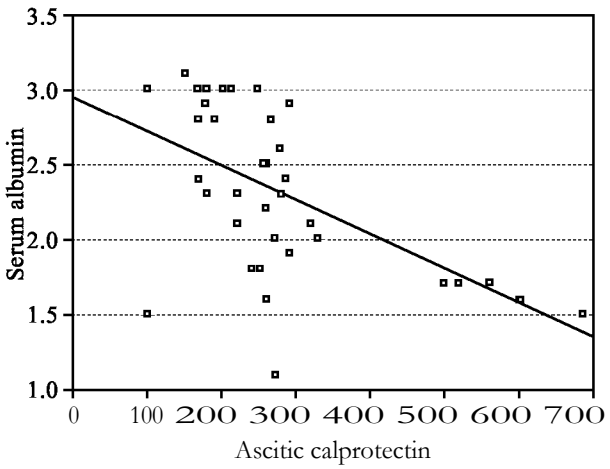

Fig. (4): Correlation between ascitic calprotectin and serum albumin level (p-value=0.001, rho=-0.716).

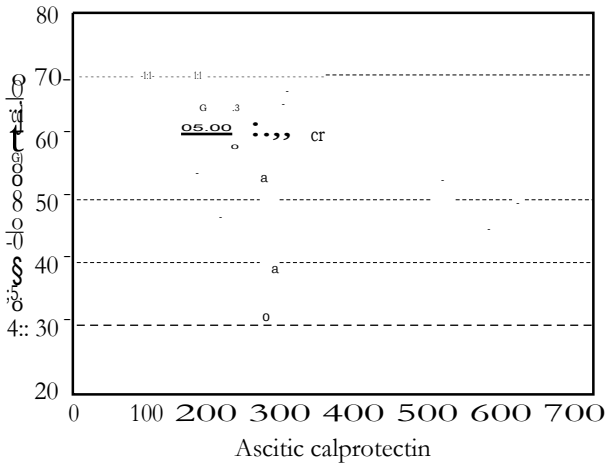

Fig. (5): Correlation between ascitic calprotectin and prothrombin conc. (p-value=0.003, rho=-0.451).

Discussion

Calprotectin is found in cells, tissues and fluids throughout the body **[13].** However, it is mainly a myelomonocytic protein constituting approximately 5% of total proteins in neutrophils **[2].**

Calprotectin is a surrogate marker of neutrophil turnover and is elevated in a number of inflammatory conditions **[13]. It** is detectable in plasma, urine, feces, cerebrospinal fluid, saliva, synovial fluid, empyema supernatant, **[13]** and colonic biopsies **[14].** Calprotectin has been studied in a variety of infective and inflammatory conditions, but more recently it has received increasing attention in gastroenterology. Its functional aspects have been reviewed recently by Johne et al. **[13].**

The emerging role for measurement of this protein in the field of gastroenterology is because of the fact that, although non-specific, it is elevated in a number of organic gastroenterological conditions. Measurement in different samples provides diagnostic and prognostic information for a number of commonly encountered organic diseases.

In the current study calprotectin was significantly higher in patients with SBP than those without SBP and this agreed with Elbanna study 1151. This increase of calprotectin can be explained by the release of calprotectin from activated neutrophil, so calprotectin had antimicrobial **[10]** and immunmodulatory actions 1111. Moreover, the elevated calprotectin correlated to severity of liver cirrhosis as there was significant positive correlation with serum bilirubin and an inverse correlation with serum albumin and prothrombin concentration. These correlations with severity of liver cirrhosis can be explained by impaired reticuloendothelial function. SBP is thought to result from a combination of factors inherent in cirrhosis and ascites, such as prolonged bacteremia secondary to compromised host defenses, intrahepatic shunting of colonized blood, and defective bactericidal activity within the ascitic fluid **[16].** Contrary to earlier theories, transmucosal migration of bacteria from the gut to the ascitic fluid is no longer considered to play a major role in the etiology of SBP **[17].**

With respect to compromised host defenses, patients with severe acute or chronic liver disease are often deficient in complement and may also have malfunctioning of the neutrophilic and reticuloendothelial systems **[18].**

As for the significance of ascitic fluid proteins, it was demonstratedthat cirrhotic patients with ascitic protein concentrations below lg/dL were 10 times more likely to develop SBP than individuals with higher concentration **[19]. It** is thought that the antibacterial, or opsonic, activity of ascitic fluid is closely correlated with the protein concentration **[20].**

Conclusion: Calprotectin might be valuable for rapid diagnosis of SBP and also for detection of severity of liver cirrhosis.

References

1- LEVISON M.E. and BUSH L.M.: Peritonitis and intraperitoneal abcesses. In Mandell G.L., Bennett J.E., Dolin R. Principles and Practiceof Infectious Diseases. 6th Edition, Elsevier, Churchill Livingstone, Philadelphia, Vol. 1, 927- 9, 2005.

2- FAGERHOL M.K., DALE I. and ANDERSSON T.: A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. Bull. Eur. Physiopathol. Respir., 16: 273-82, 1980.

3- ODINK K., CERLETTI N., BRUGGEN J., et al.: Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature, 330: 80-2, 1987.

4- WILSON G.B., JAHN T.L. and FONSECA J.R.: Demonstration of serum protein differences in cystic fibrosis by isoelectric focusing in thin-layer polyacrylamide gels. Clin. Chim. Acta., 49: 79-91, 1973.

5- WILKINSON M.M., BUSUTTIL A., HAYWARD C., BROCK D.J., DORIN J.R. and VAN HEYNINGEN V.: Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues. J. Cell Sci., 91: 221-30, 1988.

6- FREEMONT P., HOGG N. and EDGEWORTH J.: Sequence identity. Nature, 339: 516, 1989.

7- STEINBAKK M., NAESS-ANDRESEN C.F., LINGAAS E., DALE I., BRANDTZAEG P. and FAGERHOL M.K.: Antimicrobial actions of calcium binding leucocyte Ll protein, calprotectin. Lancet, 336: 763-5, 1990.

8- ROTH J., GOEBELER M. and SORG C.: S100A8 and S100A9 in inflammatory diseases. Lancet, 357: 1041, 2001.

9- BENGIS-GARBER C. & GRUENER N.: Calcium binding myeloid protein (P8,P14) is phosphorylated in fMet-Leu-Phe-stimulated neutrophils. J. Leukoc. Biol., 357: 114-18, 1993.

10- BRUN J.G., HAGA H.J., BOE E., et al.: Calprotectin in patients with rheumatoid arthritis: Relation to clinical and laboratory variables of disease activity. J. Rheumatol., 19: 859-62, 1992.

11- LEHER R.I.: Holocrine secretion of calprotectin: A neutrophil mediated defence against Candida albicans. J. Lab. Clin. Med., 121: 193-4, 1993.

12- HOMANN C., CHRISTENSEN E., SCHLICHTING P., PHILIPSEN E.K. and GRAUDAL N.A.: Ascites fluid and plasma calprotectin concentrations in liver disease-predictors of poor prognosis in cirrhosis. Gut, 49 (Suppl. III): A1163, 2001.

13- JOHNE B., FAGERHOL M.K., LYBERG T., et al.: Functional and clinical aspects of the myelomonocyte protein calprotectin. Mol. Pathol., 50: 113-23, 1997.

14- BJERKE K., HALSTENSEN T.S., JAHNSEN F., PULFORD K. and BRANDTZAEG P.: Distribution of macrophages and granulocytes expressing Ll protein (calprotectin) in human Peyer's patches compared with normal ileal lamina propria and mesenteric lymph nodes. Gut, 34: 1357-63, 1993.

15- ELBANNA A., ALLAM N., HOSSAM N., IBRAHIM A. and WAGDY M.: Plasma and ascetic floid level of calprotectin in chronic liver disease malignant and non malignantAlexandria Bulletin, 647-653, 2008.

16- RUNYON B.A. and HOEFS J.C.: "Culture-negative neutrocytic ascites: A variant of spontaneous bacterial peritonitis". Hepatology, 4 (6): 1209-11, 1984.

17- RUNYON B.A.: "Patients with deficient ascitic fluid opsonic activity are predisposed to spontaneous bacterial peritonitis". Hepatology, 8 (3): 632-637, 1988.

18- ALANIZ C. and REGAL R.E.: "Spontaneous Bacterial Peritonitis: A Review of Treatment Options". April P T 34 (4): 2, 2009.

19- RUNYON B.A.: "Low-protein-concentration ascitic fluid is predisposed to spontaneous bacterial peritonitis". Gastroenterology, December, 91 (6): 2, 1986.

20- RUNYON B.A., MORRISSEY R.L., HOEFS J.C. and WYLE F.A.: "Opsonic activity of human ascitic fluid: A potentially important protective mechanism against spontaneous bacterial peritonitis". Hepatology, 5 (4): 63, 1985.