

Association of Increased Fecal Calprotectin levels with Hepatic Encephalopathy and Spontaneous Peritonitis in Egyptian Cirrhotic Patients

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ABSTRACT

AIM: to investigate fecal calprotectin concentrations in Egyptian HCV related cirrhotic patients and recognize the relationship between these concentrations and liver cirrhosis complications as hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP).

MATERIALS AND METHODS: This was a case-control observational study, fecal calprotectin levels were checked in 100 subjects by ELISA kits; 25 cirrhotic patients experienced HE, 25 cirrhotic

patients experienced SBP, 25 cirrhotic patients without previous HE or SBP episodes in addition to 25 healthy subjects served as a control group. ROC curve analysis was used to detect the cutoff value which predicts these complications.

RESULTS: Fecal calprotectin levels were significantly elevated in cirrhotic patients and correlated with the severity of liver disease ($r = 0.64$, $p = 0.0001$). Median fecal calprotectin concentrations were significantly ($p = 0.001$) higher in HE and SBP groups than noncomplicated cirrhotic group (382.3, 358.8, 239.7mg/kg respectively). Despite that Fecal concentrations were decreased following recovery from HE, these concentrations remained significantly higher in comparison to patients without previous HE episode (292.3 versus 239.7mg/kg, $p = 0.04$), in addition, levels were more significant in patients with ascites ($p = 0.003$). ROC curve revealed that fecal calprotectin concentrations over 292.7 and 292.2mg/kg could predict patients with HE and SBP respectively, with high sensitivity and specificity.

CONCLUSION: Fecal Calprotectin has clinical value in patients with encephalopathy and SBP. Levels over 292 mg/kg may be used as a biological risk marker for SBP and HE development in Egyptian HCV related cirrhotic patients; however, further large-scale studies are needed.

Key words: Hepatic encephalopathy; Ascites; Cirrhosis; Peritonitis; Calprotectin

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INTRODUCTION

Egypt has the highest age-standardized cirrhosis mortality rates (72.7 deaths per 100,000 patients) with 18.1% of all deaths in middle aged males were due to cirrhosis^[1]. Hepatic encephalopathy (HE) and

spontaneous bacterial peritonitis (SBP) are significant complications of cirrhosis^[2].

The prevalence of HE in cirrhosis is high and can develop in up to 50% of patients^[3]. SBP presents in about 15% of patients with cirrhosis and ascites^[4] and can be diagnosed by aspiration and cytological examination of ascitic fluid but complications of the procedure may present^[5]. In some patients, the diagnosis of HE and SBP presents a substantial clinical problem as patients may present with mild cognitive impairment or not exhibit the typical features of acute peritoneal infection, so it is relevant to recognize and manage these complications in the initial stages to reduce morbidity and mortality^[6].

In cirrhosis, pathological bacterial translocation develops with a sustained increase in quantity (rate and/or degree)^[7]. The dysbiosis that occurs in the gut in patients with cirrhosis has recently been linked with complications of cirrhosis, including HE, SBP, and sepsis^[8,9].

Quantitative determination of fecal calprotectin indicates the severity of the bowel inflammation which relates to neutrophil migration into the gastrointestinal tract^[10].

We hypothesized that increased fecal calprotectin concentrations are associated with increased risk of HE and SBP in cirrhotic patients because of an increase in bacterial overgrowth and translocation; therefore, we aimed to investigate the association between fecal calprotectin concentrations and these complications and also to detect the best cutoff value for their prediction in Egyptian populations.

MATERIALS AND METHODS

Study design

A hospital-based non-interventional, case-control, observational study was carried out in Gastroenterology and Hepatology intensive care unit and inpatient wards, Internal Medicine Department in collaboration with Biochemistry Department, Faculty of Medicine, Zagazig University Hospitals.

Patient selection

The study included 75 cirrhotic patients and 25 non cirrhotic healthy subjects as control group. Diagnosis of liver cirrhosis was based on clinical, laboratory and radiological features. Eligible patients had a diagnosis of liver cirrhosis secondary to HCV infection (which is the main cause of cirrhosis in Egyptian populations). Cirrhotic patients were classified into three groups: non complicated group included 25 cirrhotic patients without a previous episode of HE or SBP, HE group included 25 patients complicated with various grades of HE, and SBP group included 25 patients complicated with SBP.

Exclusion criteria

We excluded patients with liver cirrhosis secondary to causes other than HCV infection, patients with other causes of elevated fecal calprotectin as; inflammatory bowel disease, Celiac disease, colorectal carcinoma, patients with infectious colitis, dysentery or diarrhea with fever, patients with recent GIT bleeding and patients refuse to participate in the study. We also excluded any patients who previously received NSAIDs or recent P.P.I therapy. Patients with other causes of altered sensorium due to metabolic or neurological deficit and patients with combined (HE and SBP) were also excluded.

The Child-Pugh classification of patients was performed as well as Child's score calculated for each patient^[11]. All cirrhotic patients with ascites underwent diagnostic abdominal paracentesis to obtain an ascitic fluid sample for laboratory investigation.

Diagnosis of HE was performed coinciding with West Haven crite-

ria^[12]. Diagnosis of SBP was done by measuring the total leukocytic count in ascitic fluid samples. Presence of > 250 polymorph nuclear cells (PMN) /mm³ in the ascitic fluid and positive ascitic fluid bacterial culture without an intra-abdominal surgically treatable source of infection was used for diagnosis^[13,14].

Methods

All patients in the current study were subjected to through history taking, including the history of previous episodes of HE or SBP and clinical examination with an assessment of HE grades.

Laboratory assessment

About 1-5 g stool was collected and placed in a suitable container and stored at 20° C. Frozen samples were allowed to reach room temperature. About 100 mg (between 40 - 120 mg) were placed into a screw-cap-tube. A europsital stool collection device containing the amount of extraction solution was used; it allowed collection of the amount of stool desired for the calprest assay directly from the stool container without weighing the sample.

Samples were shaken for 30 seconds by a vortex. The homogenate (1 ml) was transferred to an Eppendorf tube and centrifuged for 20 min at 10.000g at RT using a bench-top centrifuge. 0.5 ml of the clear extract supernatant was transferred to a new Eppendorf tube and stored at -20°C. Calprotectin was measured by ELISA consuming (CALPREST) kits manufactured by (Eurospital, Trieste, Italy) at the Central Laboratory of Zagazig University Hospital. The concentration of calprotectin in the samples is calculated employing the standards provided.

All subjects underwent routine laboratory testing, including liver and renal function tests, C.B.C, inflammatory markers as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and coagulation profile. Also, pelviabdominal ultrasonography was done for all patients for confirmation of liver cirrhosis and detection of ascites or focal hepatic lesions.

Statistical analysis

Data were collected tabulated and statistically analyzed using SPSS 20.0 for Windows (SPSS Inc., Chicago, USA) and MedCal (MedCal 10 software, Ostend, Belgium). Quantitative data were expressed as the mean ± SD and /or as median (range), and qualitative data were expressed as numbers and percentages. Data were tested for normal distribution using the Shapiro Walk test.

The Mann Whitney U test was used to compare fecal calprotectin concentrations between patients recently recovered from HE and non complicated group, patients with and without ascites, ascetic patients with and without SBP. F-test was used to compare between groups as regards Child's score, hemoglobin, INR, total bilirubin, WBC's, albumin, ESR and creatinine mean ± SD. The least significant difference (LSD) was used to detect significance between groups while, Kruskal-Wallis test was used for detection of the significant difference between groups as regards AST, ALT and fecal calprotectin concentrations. The Chi-square test was used for gender and Child's class.

The Spearman correlation coefficient was used when appropriate. Receiver Operating Characteristic (ROC) curve analysis was used for calculation of the optimal cutoff value of fecal calprotectin with maximum sensitivity and specificity for predicting HE and SBP using the Youden index.

All tests were two-sided. P-value <0.001 was considered statistically highly significant, P-value < 0.05 was considered statistically significant.

RESULTS

Basic subject data were summarized in table 1.

Median Fecal calprotectin concentrations were significantly higher in cirrhotic patients in comparison to control group ($p < 0.001$). HE and SBP groups had higher fecal calprotectin concentrations (382.3 & 358.8 mg/kg respectively) compared to noncomplicated cirrhotic group (239.7 mg/kg) with a highly significant difference ($p = 0.001$); Table 2, Figure 1. More Advanced Child’s classes C and B had higher significant median fecal calprotectin concentrations compared to Child’s class A (443.8, 271.6, 91.6 mg/kg, respectively, $p = 0.001$) with a highly significant direct correlation between these concentrations and Child’s score ($r = 0.64, p = 0.0001$); Table 2, Figure 2.

Higher fecal concentration was associated with progressive stages of HE but without statistical significance ($p > 0.05$), however, patients recently recovered from HE following treatment experienced higher median concentrations than patients without a previous episode of HE (292.3 versus 239.7mg/kg, $p = 0.04$); Table 2. Patients

recently recovered from HE following treatment had lower fecal concentrations than patients with HE but without statistical significance ($p > 0.05$).

More significant median fecal concentrations were found in patients with ascites than patients without in noncomplicated cirrhotic group (250.7 versus 88.9 mg/kg, $p = 0.003$); also significant higher concentrations were found in patients with SBP compared to ascetic patients without SBP in noncomplicated group (358.8 versus 250 mg/kg, $p = 0.01$); Table2. No statistically significant correlation between ESR, CRP, total leucocytic count (TLC) in ascetic fluid and fecal calprotectin in cirrhotic patients with SBP was detected ($r = 0.15, 0.17, 0.24$ respectively, $p > 0.05$).

ROC curve analysis revealed that fecal caproprotectin levels over 292.7 could predict hepatic encephalopathy in cirrhotic patients with 80% sensitivity and 80% specificity, Figure 3. Furthermore, the cutoff level over 292.2 could predict patients with SBP with 72% sensitivity and 80% specificity, Figure 4. The areas under the curve were 0.82 and 0.74 respectively.

Table 1 Demographic, clinical and laboratory data of enrolled subjects.

	Control (N=25)	Non complicated cirrhosis (N=25)	HE (N=25)	SBP(N=25)	P value
Age (years)	55.7 ± 6.2	57.8 ± 8.6	57.9 ± 5	54.6 ± 8	0.2 †
Gender (M/F)	15/10	17/8	10/15	11/14	0.1 §
Males/female ratio	1.5	2.1	0.67	0.79	
Child's class					
A	-	6 (24)	-	3 (12)	0.007* §
B	-	14 (56)	8(32)	11 (44)	
C	-	5 (20)	17(68)	11(44)	
Child's score	-	8 ± 2	10.6 ± 2	9.2 ± 1.8	0.001* †
Hemoglobin (g/dl)	13 ± 0.9	10.4 ± 1.8	10.7 ± 2	10.4 ± 1.4	0.000** †
WBC's (×10 ³ /cm)	7 ± 2.2	6.2 ± 1.8	6.5 ± 3.5	8 ± 3.8	0.15†
INR	1.1 ± 0.0	1.4 ± 0.34	1.5 ± 0.26	1.6 ± 0.36	0.000** †
Serum AST (U/L)	20 (14-40)	32 (15-180)	35 (18-150)	42.3 (10.1227)	0.59 ‡
Serum ALT (U/L)	18.9 (17-39)	24 (12-95)	31 (10-445)	32 (2.4-196)	0.97 ‡
Total bilirubin (mg/dl)	1.1 ± 0.1	1.8 ± 0.7	3.3 ± 2	3.2 ± 3.2	0.000** †
Albumin (g/dl)	3.9 ± 0.4	2.9 ± 0.75	2.6 ± 0.46	2.7 ± 0.9	0.000** †
ESR	12 ± 3.6	38.5 ± 12	47.2 ± 17	64.6 ± 18	0.000** †
Creatinine (mg/dl)	1.2 ± 0.2	1.2 ± 0.34	1.3 ± 0.8	1.2 ± 0.6	0.88†

Quantitative data were expressed as mean ± SD except AST and ALT were expressed as median (range), while, qualitative data were expressed as numbers and percentages. †F test, ‡Kruskal-Wallis test, § Chi-square test. WBC's, white blood cells count; INR, international normalizing ratio; ALT, alanine transaminase; AST, aspartate transaminase; ESR, erythrocyte sedimentation rate, * significant value, ** high significant value.

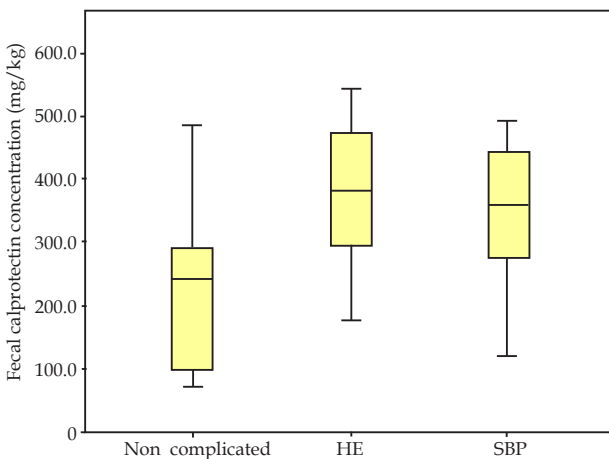


Figure 1 The box - plot diagram represents the range of fecal calprotection among the 3 cirrhotic groups ($p = 0.001$); the upper & the lower line in each box represent the 75th & 25th percentile respectively while the line through each box indicates the median. Whiskers represent the range between the minimum and maximum values.

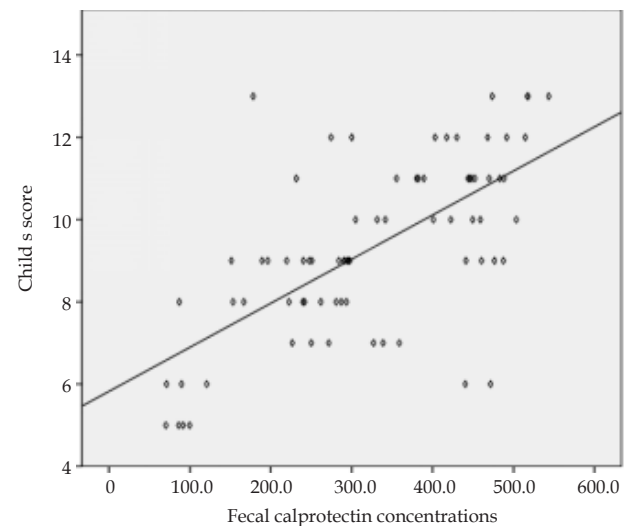


Figure 2 Correlation of fecal calprotectin concentrations with Child’s score in the considered groups ($r 0.64, p = 0.001$)

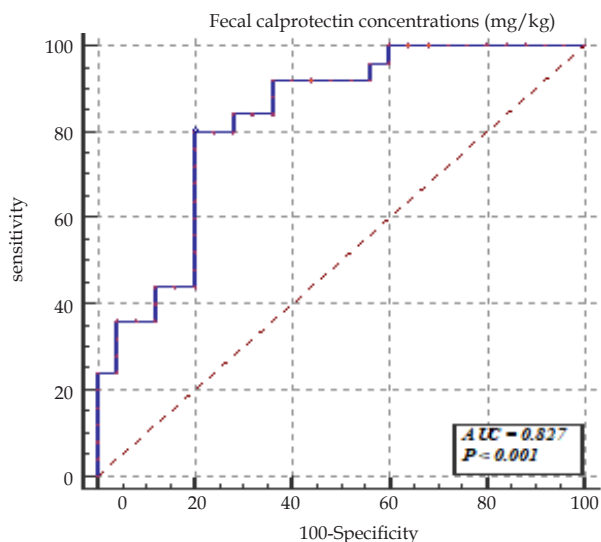


Figure 3 Roc curve for the best optimal cut-off value of fecal calprotectin for diagnostic performance in HE patients (AUC 0.82, 95% CI 0.7-0.94, $p < 0.001$, optimal cut-off 292.7, sensitivity 80%, specificity 80%, PPV 80%, NPV 80%, accuracy 80%).

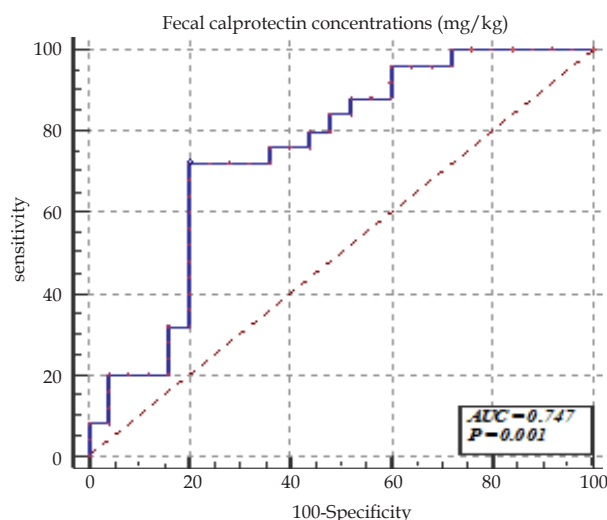


Figure 4 Roc curve for the best optimal cut-off value of fecal calprotectin for diagnostic performance in SBP (AUC 0.74, 95% CI 0.61-0.89, $p = 0.001$, optimal cut-off 292.2, sensitivity 72%, specificity 80%, PPV 78%, NPV 74%, accuracy 76%).

DISCUSSION/CONCLUSION

Over the preceding years, fecal calprotectin had been investigated as a biological marker of intestinal inflammation, especially in inflammatory bowel disease and less often in liver cirrhosis, so this study was designed with the aim to investigate the association between fecal calprotectin concentrations and the complications of HCV-related cirrhosis, particularly SBP and HE in Egyptian populations.

Fortunately, to our knowledge, few studies addressed this issue in Egyptian cirrhotic patients related to HCV, which is the main cause of cirrhosis in Egypt.

In the current study, we confirmed that fecal calprotectin concentrations were significantly elevated in cirrhotic patients and this elevation was significantly correlated with the severity of liver disease as assessed by Child-Pugh class and score. We found similar observations in Gundling *et al.* study^[15].

In line with current results Yagmur *et al*^[16] found elevated fecal calprotectin in patients with progressive disease and more excessive concentrations in subjects with alcoholic cirrhosis and these fecal concentrations depend on the severity of liver disease. Pleguezuelo *et al*^[17] were equally in agreement with our results, they found that calprotectin level in cirrhotic patients was elevated in faces rather than in plasma.

These results confirm the hypothesis that cirrhotic patients are more susceptible to bacterial infections because of increased migration of bacteria or bacterial products from the intestinal lumen owing to the hepatic and reticuloendothelial system dysfunction^[18].

Small intestinal bacterial overgrowth frequently occurs in decompensated cirrhosis and correlates with systemic endotoxemia^[19]. Small intestinal dysmotility and altered bile composition in cirrhosis contribute to bacterial overgrowth and the development of endotoxemia and infectious complications^[20].

Once a systemic or local immune response has been established, cytokines like tumor necrosis factor (TNF) and interleukin (IL)-6 disrupt the intestinal barrier integrity by altering the structure of the apical junctional complex, further increasing intestinal mucosal permeability that observed in cirrhosis^[21].

In the current study, we found higher concentrations of calprotectin

Table 2 Fecal calprotectin concentrations (mg/kg).

	N	Mean	Median (range)	P value
Control	25	24.4	25.7 (10-50)	0.000** †
Non complicated cirrhosis	25	236.4	239.7 (70.3-483.2)	
HE	25	387	382.3 (178.2-543.4)	
SBP	25	351.4	358.8 (120.8-491.5)	
Child's class				
A	9	171.2	91.6 (70.3-471.5)	0.001* †
B	33	276.3	271.6 (86.9-487.4)	
C	33	415.5	443.8 (178.2-543.4)	
Encephalopathy grade				
Recent recovery (0) §	6	326.7	292 (261.8-503.3)	0.28 †
1	5	381	382 (178.19-517.1)	
2	6	394.3	442.7 (222.5-488.2)	
3	8	430.5	461.5 (294.4-543.4)	
Recently recovered	6	326.7	292.3 (261.8-503.3)	0.04* †
Non complicated cirrhosis	25	236.4	239.7 (70.3-483.2)	
Ascites (in noncomplicated cirrhosis)				
No	6	111.5	88.9 (70.3-250.04)	0.003* †
Yes	19	276	250.7 (87-483.2)	
SBP				
Yes	25	351.4	358.8 (120.8-491.5)	0.01* †
No ¶	19	276	250.7 (87- 483.2)	

† Kruskal-Wallis test, ‡ Mann Whitney U. § Patients were considered grade 0 according to normal consciousness, behavior and neurological findings following recovery from hepatic encephalopathy. ¶ cirrhotic patients with ascites not complicated with SBP in noncomplicated group, * significant value, ** high significant value.

in patients with HE compared to noncomplicated cirrhotic group ($p = 0.0001$). Because of this highly significant difference, we tried to provide a cutoff value for calprotectin to be used in discriminating patients with HE from patients without a previous episode of HE and we found the best cutoff value of 292.7mg/kg demonstrated 80% sensitivity, 80% specificity and 80% positive predictive value with AUC 0.82. Gundling *et al*^[15] used cut point of 164 mg/kg in differentiating gradings of 0 and 1 from 2 and 3 but not for discriminating patients with HE from cirrhotic patients without previous episodes.

In line with current results, a single Egyptian study by Mohamed *et al*^[22] found a cut off value of 280 mg/kg with AUC of 0.72 for diagnosing HE.

On top, we found patients recently recovered from HE had statistically significantly higher concentrations of calprotectin than noncomplicated group ($p = 0.04$). These findings may provide a notable clinical application in the detection of the initial stages of HE (minimal HE) when grading of the severity of HE may be subjective using only clinical criteria. Gupta and colleagues^[23] were in accordance with our results as they showed that the frequency of bacterial overgrowth is high in patients with minimal HE. These findings may confirm the hypothesis that bacterial translocation from the gut lumen plays a key role in the development of HE and represent a responsible factor for minimal HE in these patients^[24].

A significant correlation between elevated fecal calprotectin and HE grades as measured by West–Haven criteria were detected in Gundling *et al* study^[15] and calprotectin concentrations were significantly correlated with serum ammonia levels. Alempijević *et al*^[24] found that fecal calprotectin concentrations were positively correlated with HE grades but didn't establish a correlation with serum ammonia levels as in Gundling *et al* study^[15].

The results of these previous studies were near to our results as there were more excessive median fecal concentrations in patients with progressive stages of HE (461.5 mg/kg for grade 3 versus 292 mg/kg for grade 0) but this didn't reach a statistically significant value ($p > 0.05$) probably because of the more limited sample size in the current study.

Significantly higher concentrations of fecal calprotectin in patients with ascites were found compared to patients without ascites in noncomplicated group, Gundling *et al*^[15] reported also in his study that median fecal calprotectin was higher when ascites was present, but this difference was not significant ($p = 0.7$). Patients with ascites have advanced Child's class than patients without and our results previously reported a significant direct correlation between calprotectin and Child's class.

More elevated significant concentrations of fecal calprotectin were detected in patients with SBP compared to all patients in noncomplicated group ($p = 0.003$) and to ascetic patients without SBP in the same group ($p = 0.01$); a cutoff value 292.2 mg/kg was found to have 72% sensitivity, 80% specificity and 78% positive predictive value in diagnosing patients with SBP with AUC 0.74.

These results were in accordance with similar results found in Yagmur *et al*^[16] and Gundling *et al*^[15] studies. In current study higher cutoff value for diagnostic performance of SBP in our populations was detected compared to a low cutoff value of 140 mg/kg with 71% sensitivity and 79% specificity in Gundling *et al*^[15] The difference in cutoff value may be due to varying causes of cirrhosis (viral hepatitis versus the alcoholic); they confirmed different values of fecal calprotectin according to different aetiologies (Median FCCs (mg/kg) were as follows: 67.15 in alcoholic, 111.15 in HCV, 243.25 in HBV, 1.5 in autoimmunehepatitis and 35.3 in cryptogenic cirrhosis) and these results also addressed the ethnicity and environmental differences.

There was no statistically significant correlation between ESR, CRP, total leucocytic count in ascetic fluid and fecal calprotectin concentrations in cirrhotic patients with SBP, Gundling *et al*^[15], also found no significant relation between laboratory variables of systemic inflammation (CRP, WBC count) and fecal calprotectin ($p = 0.14$, 0.2 respectively). Elevated fecal calprotectin in cirrhotic patients is related to local bacterial translocation and not due to a systemic inflammatory reaction which may be the explanation of these results.

In conclusion, fecal Calprotectin may be a key marker for bacterial translocation in cirrhosis; it is a non-invasive, quick and relatively easy to perform assay with proven clinical value in Egyptian patients complicated with encephalopathy and SBP in the current study. Concentrations over 292 mg/kg may be used as a biological risk marker for SBP and HE development in Egyptian cirrhotic patients; however, less expensive testing that makes its use more sense in daily practice need to be present.

While the clinical signs of higher grades of encephalopathy are evident and no cost-effect of fecal calprotectin compared to PMN count in the ascetic fluid for diagnosis of SBP is present, testing for fecal calprotectin may provide a value when mild cognitive impairment (early encephalopathy) is present or the typical features of peritonitis are absent and to put appropriate primary prophylactic measures.

Limitations of study

Limitations of this study are the limited number of patients included and single center experience, so in future large-scale studies from multi-centers in Egypt should be conducted to confirm this conclusion based on these preliminary data. Additionally, there was a defect in performing psychomotor testing to differentiate between patients with minimal hepatic encephalopathy (MHE) and patients with complete recovery and if there is a difference between them as regards fecal calprotectin concentrations.

Statement of Ethics

The study was conducted in accordance with the ethical standards of the institutional review board and with the Declaration of Helsinki (ZU-IRB#: 3422-14-2-2017). Written informed consent was obtained from all participants included in the study or from first degree relatives of patients with altered sensorium.

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Author Contributions

Yasser A. Elnaggar: conception, design of the study and critical revision of the article for important intellectual content. Haidy E. Zidan: interpretation of the data and critical revision of the article for important intellectual content. Elham M. Abdelbaky: analysis, interpretation of the data and drafting of the article. Fady M. Wadea: conception, design of the study; analysis and interpretation of the data; drafting of the article; critical revision of the article for important intellectual content. All authors made the final approval of the article.

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