European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 09, Issue 06, 2022

Original Research Article

The correlation of fecal calprotectin with severity of liver cirrhosis and hepatic encephalopathy

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Abstract

Regular use of anti-inflammatory agents (NSAIDS) may cause enteropathy in some individuals influencing FC concentration; therefore, it is recommended to stop NSAIDS several weeks before FC stool measurement. Calprotectin is measured by ELISA assays and a number of commercial kits are available on the market. During the Study period approximately 200 to 250 cases satisfying the Inclusion and Exclusion criteria were expected in each group. So after selecting the first eligible case in each Group by simple random procedure, every 10th eligible case in that Group have been enrolled till the required sample size of 22 cases in each Group was achieved. There was statistically significant difference in fecal calprotectin concentration between CTP class A and C, between class B and C. Though mean fecal calprotectin concentration was high in CTP class B as compared to class A, the difference was not statistically significant.

Keywords: Fecal calprotectin, severity of liver cirrhosis, hepatic encephalopathy

Introduction

Calprotectin is a calcium-binding protein (heterodimer S100A8/A9), classified as damage associated molecular pattern protein, having antimicrobial properties. It accounts for 60% of neutrophil cytosolic content and it is also found in monocytes and macrophages. The accumulation of neutrophils in the mucosa, a feature of inflammatory flares, results in the release of FC in the stools, where it can be easily measured. FC excretion has been shown to correlate well with 111 Indium test. Calprotectin is not subject to proteolytic degradation in feces and remains stable at room temperature for 3-7 days. The variability inevitably found in calprotectin concentration does not reduce its clinical utility and no dietary substance has been found to interfere with the assays [1].

Regular use of anti-inflammatory agents (NSAIDS) may cause enteropathy in some individuals influencing FC concentration; therefore, it is recommended to stop NSAIDS several weeks before FC stool measurement. Calprotectin is measured by ELISA assays and a number of commercial kits are available on the market. Several studies have evaluated the semi quantitative and qualitative point-of-care assays and the results are comparable to that of

ISSN 2515-8260 Volume 09, Issue 06, 2022

ELISA testing.

The normal range for FC is considered <50 μg/g of feces; however, there is a great variability in healthy population depending on patients' age. Calprotectin level is higher in pediatric age, that is, before 5 years (when it starts aligning with adults values), probably because of an increased permeability in intestinal mucosa and differences in intestinal flora. FC for children from 2 to 9 years is considered normal if <166 μg/g, in adults aged between 10 and 59 if <51 μg/g and after 60 years <112 μg/g (113) [2].

The pathogenesis of bacterial translocation in patients with cirrhosis has been associated with alterations in gut mucosal immune responses and intestinal permeability. In addition, neutrophil infiltrates are detected in the gastrointestinal mucosa of cirrhotics. Consequently, calprotectin has been investigated as a possible diagnostic marker for the existence and natural history of SBP and HE [3].

Initial studies conducted by a Danish research group, focused on the possible prognostic significance of calprotectin levels in the plasma and ascitic fluid samples from patients with end-stage liver disease. The authors did not find a significant difference between healthy controls and patients with cirrhosis (irrespectively if liver disease was compensated or decompensated), a finding that was confirmed in additional studies. On the other hand, they reported that high plasma calprotectin levels were an indicator of poor survival in alcohol-related cirrhosis. The most important finding, however, regarding the role of calprotectin in relation to bacterial translocation, was that during follow up of the patients higher calprotectin levels were an independent predictor of recurrent bacterial infections [4].

The most important study regarding the role of fecal calprotectin in the diagnosis of bacterial translocation complications, is the one conducted by Gundling *et al*. They investigated the relationship between fecal calprotectin levels and the onset and course of SBP and HE. They confirmed that patients with cirrhosis had significantly elevated fecal calprotectin levels when compared to healthy controls. Moreover, this increase correlated with the severity of liver disease (assessed by Child-Pugh and MELD scores). Even more significantly, higher calprotectin values were associated with advanced stages of HE, the presence of SBP, as well as extra intestinal infections. Finally, calprotectin strongly correlated with serum ammonia levels. The aforementioned observations reinforced the hypothesis that fecal calprotectin may be a reliable surrogate marker for bacterial translocation and provide important assistance in the diagnosis and clinical management of patients with decompensated liver disease [5].

It was recently reported that ascitic calprotectin may be utilized (with the help of a point-of-care assay) to reliably predict an elevated polymorphonuclear count (> 250) in ascitic fluid, allowing for faster diagnosis of SBP.

Another study by Alempijević *et al.* focusing exclusively on HE confirmed that fecal calprotectin levels were positively correlated with HE grading according to the West-Haven grouping criteria, although it did not show a correlation with serum ammonia levels as Gundling *et al* did.

The ratio of ascites calprotectin to total protein was proposed as a better marker than ascitic fluid calprotectin alone for use in the diagnosis and prognosis of SBP. The authors report satisfactory sensitivity and specificity for this new marker, as well as a statistically significant correlation of higher values with poor 30-d survival [6].

In all, calprotectin remains a promising surrogate marker for bacterial translocation in cirrhosis. It demonstrates many advantages, especially in its fecal measurement, as it is a non-invasive, quick and relatively easy to perform assay, with proven clinical value in other disease states.

ISSN 2515-8260 Volume 09, Issue 06, 2022

Methodology

Inclusion criteria

1. Age > 18 years.
2. Cirrhosis of liver with or without hepatic encephalopathy.

Exclusion criteria

1. Patients with Suspected Inflammatory Bowel Disease.
2. Patients with Gastro Intestinal bleed.
3. Patients on Proton Pump Inhibitors.
4. Patients on Non-steroidal anti-inflammatory drugs.
5. Patients with Diarrhea.

Sampling procedure

We had selected 22 cases of liver cirrhosis without hepatic encephalopathy (Group A) and 22 cases of liver cirrhosis with hepatic encephalopathy (Group B).
During the Study period approximately 200 to 250 cases satisfying the Inclusion and Exclusion criteria were expected in each group. So after selecting the first eligible case in each Group by simple random procedure, every 10th eligible case in that Group have been enrolled till the required sample size of 22 cases in each Group was achieved.

Methods of measurement of outcome of interest and data collection methods

Diagnosis of liver cirrhosis was based on clinical clues from the patient's medical history, physical examination, laboratory tests, abdominal ultrasonography and CT abdomen.
The degree of liver insufficiency was assessed by the Child-Pugh classification and Model of End Stage Liver Disease-Sodium (MELD Na). Hepatic encephalopathy staging was done according to West-Haven criteria.
Fecal calprotectin assay was done in patients of both the groups using Chemi Luminiscence Immuno Assay (CLIA). Calprotectin values <50 mcg/gm of feces was considered as normal and not indicative of inflammation in the gastrointestinal tract.
A "Patient information sheet" (Appendix-C) in English and also in local language was handed over to the patient prior to inclusion in the study. All patients gave written consent prior to inclusion in the study. The data collected was summarized using tables and charts.

Results

Table 1: Mean fecal cal protect in in group A and group B

	Mean Fecal Calprotectin (mcg/gm)
Group A	40.47± 14.55
Group B	184.48± 92.63

Mean fecal calprotectin in group A was 40.47 ± 14.55 mcg/gm and in group B was 184.48 ± 92.63 mcg/gm.

Table 2: Distribution of patients according to CTP class in both groups

CTP	Group A	Group B
Class A	9 (40.9%)	2 (9.1%)
Class B	10 (45.5%)	7 (31.8%)
Class C	3 (13.6%)	13 (59.1%)

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In group A, 9 (40.9%) of patients belonged to CTP class A, 10 (45.5%) belonged to class B and 3 (13.6%) belonged to class C. In group B 2 (9.1%) belonged to CTP class A, 7 (31.8%) belonged to class B and 13 (59.1%) belonged to class C.

Table 3: Distribution of patients according to W-H grade of HE

W-H Grade	Group B
I	31.8%
II	36.4%
III	31.8%

In group B 31.8% of patients belonged to W-H grade I HE, 36.4% to grade II and 31.8% to grade 3 HE.

Table 4: Mean fecal calprotectin in different grades of HE

HE Grade	Mean Fecal Calprotectin (mcg/gm)
W-H GR I	84.214± 11.98
W-H GR II	167.788± 20.49
W-H GR III	303.814± 26.11

Mean fecal calprotectin concentration in grade I HE patients was 84.21 ± 11.98 mcg/gm, in grade II 167.79 ± 20.49 mcg/gm and in grade III 303.81 ± 26.11 mcg/gm.

Table 5: Mean FC according to CTP class in both groups

	Mean Fecal Calprotectin (mcg/gm)		
	CTP-A	CTP-B	CTP-C
Group A	29.42	45.72	56.10
Group B	70.90	121.03	236.12

In group A mean fecal calprotectin of CTP class A was 29.42 mcg/gm, class B was 45.72 mcg/gm and class C was 56.10 mcg/gm.
In group B mean fecal calprotectin of CTP class A was 70.90 mcg/gm, class B was 121.03 mcg/gm and class C was 236.12 mcg/gm.

Table 6: Correlation of FC with age and MELD Na score

		Fecal Calprotectin
Age	Pearson Correlation(r)	-.129
	Sig. (P-value)	.405
	N	44
MELD_Na	Pearson Correlation(r)	.545
	Sig. (P-value)	.000
	N	44

p<0.05-significant.

Pearson correlation statistical test showed that there was no significant correlation between age and fecal calprotectin concentration with p = 0.405.
Pearson correlation statistical test showed that there was significant correlation between fecal calprotectin concentration and severity of liver cirrhosis according to MELD Na with p value < 0.001.

Table 7: Multiple Comparisons between FC and grades of HE

(I) W_H_grade	(J) W_H_grade	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-83.5732*	10.5466	.000	-111.259	-55.887
	3	-219.6000*	10.8925	.000	-248.194	-191.006
2	1	83.5732*	10.5466	.000	55.887	111.259
	3	-136.0268*	10.5466	.000	-163.713	-108.341
3	1	219.6000*	10.8925	.000	191.006	248.194
	2	136.0268*	10.5466	.000	108.341	163.713

*. The mean difference is significant at the 0.05 level.

The above table shows the mean differences in fecal calprotectin levels between different grades of hepatic encephalopathy. Significant difference between values of fecal calprotectin was found between different grades of hepatic encephalopathy according to West Haven grading.

Table 8: Multiple Comparisons between FC and CTP classes (Both groups combined)

(I) CPS	(J) CPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-39.7658	26.9600	.444	-107.063	27.532
	C	-165.3989*	27.2890	.000	-233.518	-97.280
B	A	39.7658	26.9600	.444	-27.532	107.063
	C	-125.6331*	24.2680	.000	-186.211	-65.055
C	A	165.3989*	27.2890	.000	97.280	233.518
	B	125.6331*	24.2680	.000	65.055	186.211

*. The mean difference is significant at the 0.05 level.

The above table shows the mean differences in fecal calprotectin between different CTP classes in Group A and B combined. Significant difference between values of fecal calprotectin was found between CTP class A and C and also between class B and class C. Though the mean fecal calprotectin was higher among class B patients as compared to class A it was not statistically significant.

Table 9: Multiple Comparisons between FC and CTP classes (Group A)

(I) CPS	(J) CPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-16.2978*	5.0951	.014	-29.673	-2.923
	C	-26.6778*	7.3927	.006	-46.084	-7.271
B	A	16.2978*	5.0951	.014	2.923	29.673
	C	-10.3800	7.2997	.514	-29.542	8.782
C	A	26.6778*	7.3927	.006	7.271	46.084
	B	10.3800	7.2997	.514	-8.782	29.542

*. The mean difference is significant at the 0.05 level.

The above table shows the mean differences in fecal calprotectin between different CTP classes in Group A. Significant difference between values of fecal calprotectin was found between CTP class A and B and also between class A and class C. Though the mean fecal calprotectin was higher among class C patients as compared to class B it was not statistically significant.

Table 10: Multiple Comparisons between FC and CTP classes (Group B)

(I) CPS	(J) CPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-50.1286	55.6503	1.000	-196.216	95.959
	C	-165.2154*	52.7192	.016	-303.609	-26.822
B	A	50.1286	55.6503	1.000	-95.959	196.216
	C	-115.0868*	32.5390	.007	-200.505	-29.668
C	A	165.2154*	52.7192	.016	26.822	303.609
	B	115.0868*	32.5390	.007	29.668	200.505

*. The mean difference is significant at the 0.05 level.

The above table shows the mean differences in fecal calprotectin between different CTP classes in Group B. There was statistically significant difference in fecal calprotectin concentration between CTP class A and C, between class B and C. Though mean fecal calprotectin concentration was high in CTP class B as compared to class A, the difference was not statistically significant.

Discussion

In the present study, in patients with hepatic encephalopathy, 31.8% of patients belonged to WH grade I HE, 36.4% of patients belonged to WH grade II HE and 31.8% of patients belonged to WH grade III HE. In a study by Inas Elkhedr Mohamed *et al*. [7], 37.5% of patients belonged to WH grade I HE, 33.3 % of patients belonged to WH grade II HE and 29.2% of patients belonged to WH grade III HE.

In the present study, in group A mean fecal calprotectin of CTP class A was 29.42 mcg/gm, class B was 45.72 mcg/gm and class C was 56.10 mcg/gm. In group B mean fecal calprotectin of CTP class A was 70.90 mcg/gm, class B was 121.03 mcg/gm and class C was 236.12 mcg/gm. In the study by Inas Elkhedr Mohamed *et al*. [8], the mean fecal calprotectin concentration in CTP class A was 66±15 mcg/gm, in class B it was 116±12 mcg/gm. In a study by Salem *et al*. [8] the mean fecal calprotectin in class A, class B and class C were 52.19±16.99 mcg/gm, 182.5±74.81 mcg/gm and 311.47±90.92 mcg/gm respectively. In the above studies the mean FCC were found to be higher as the severity of cirrhosis increased as assessed by CTP score.

In the present study the overall mean difference in fecal calprotectin concentration between CTP class A and class B was 39.76 with standard error of 26.96 which was not statistically significant with p value of 0.444. The mean difference in fecal calprotectin concentration between CTP class B and class C was 125.63 with standard error of 24.26 which was statistically significant with p value of 0.001. The mean difference in fecal calprotectin concentration between CTP class A and class C was 165.39 with standard error of 27.28 which was statistically significant with p value of 0.001.

In the present study in group A the mean difference in fecal calprotectin concentration between CTP class A and class B was 16.29 with standard error of 5.09 which was statistically significant with p value of 0.014. The mean difference in fecal calprotectin concentration between CTP class B and class C was 10.38 with standard error of 7.29 which was not statistically significant with p value of 0.514. The mean difference in fecal calprotectin concentration between CTP class A and class C was 26.67 with standard error of 7.39 which was statistically significant with p value of 0.006.

In the present study in group B the mean difference in fecal calprotectin concentration between CTP class A and class B was 50.12 with standard error of 55.65 which was not statistically significant with p value of 1.0. The mean difference in fecal calprotectin concentration between CTP class B and class C was 115.08 with standard error of 32.53

ISSN 2515-8260 Volume 09, Issue 06, 2022

which was statistically significant with p value of 0.007. The mean difference in fecal calprotectin concentration between CTP class A and class C was 165.21 with standard error of 52.71 which was statistically significant with p value of 0.016.

In the present study, mean fecal calprotectin in group A was 40.47±14.55 mcg/gm and in group B was 184.48±92.63 mcg/gm, where as in a study by Amany Lashin *et al*. [9] mean fecal calprotectin was 57.55±8.92 mcg/gm in cirrhosis patients without HE and 304.4±41.05 mcg/gm in patients with HE. In both the studies mean fecal calprotectin was higher in cirrhotic patients with HE.

In the present study, mean fecal calprotectin concentration in WH grade I HE patients was 84.21 ± 11.98 mcg/gm, in grade II was 167.79 ± 20.49 mcg/gm and in grade III was 303.81 ± 26.11 mcg/gm. In a study by Inas Elkhedr Mohamed *et al*. [7] mean fecal calprotectin concentration in WH grade I HE patients was 239±19 mcg/gm, in grade II was 430±11 mcg/gm and in grade III was 468±14 mcg/gm. In another study by Tamara Alempijevic *et al*. [10], mean fecal calprotectin in WH grade 1 was 156±143 mcg/gm and in grade II was 380.7±107.4 mcg/gm. In all the above studies it was found that patients with higher grades of West Haven classification for HE had higher mean fecal calprotectin.

In the present study the mean difference in fecal calprotectin concentration between grade I and grade II hepatic encephalopathy was 83.57 with standard error of 10.54 and was statistically significant with p value of <0.001. The mean difference in fecal calprotectin concentration between grade II and grade III hepatic encephalopathy was 136.02 with standard error of 10.54 which was statistically significant with p value of 0.001. The mean difference in fecal calprotectin concentration between grade I and grade III hepatic encephalopathy was 219.60 with standard error of 10.89 which was statistically significant with p value of 0.001. The mean fecal calprotectin was within normal value in patients with liver cirrhosis without hepatic encephalopathy in CTP class A and B, but it was elevated in CTP class A, B and C in liver cirrhosis with hepatic encephalopathy group.

The prevalence of HE in cirrhosis is presumably high and can be diagnosed in up to 80% of all cirrhotic patients (126). Compared with, e.g. ascites or oesophageal variceal bleeding, HE seems to represent an often overlooked complication in cirrhotic patients. Several diagnostic systems have been used to diagnose the severity of HE clinically (e.g. West-Haven criteria) and technically using objective techniques such as CFF. Despite growing knowledge concerning newer neuroimaging modalities including magnetization transfer imaging or proton magnetic resonance spectroscopy, it has to be emphasized that the possibilities to diagnose HE in everyday practice are limited to only few feasible methods.

The GI tract of cirrhotic patients shows various alterations of its mucosal barrier including infiltrates of neutrophils. Cirrhotic patients are in particular susceptible to bacterial infections because of increased migration of bacteria or bacterial products from the intestinal lumen related to liver dysfunction and reduced reticuloendothelial function. Bacterial overgrowth is common ranging between 30 until 64% and seems to represent one of the main factors to trigger bacterial translocation. A study demonstrated that bacterial overgrowth is a responsible factor for minimal HE in cirrhotic patients. Altered gut flora and bacterial translocation are known to play an important role in the pathogenesis of complications of cirrhosis such as HE and SBP. Calprotectin in cirrhotic patients can be considered as a valid marker of intestinal inflammation. Qualities of calprotectin such as protein stability up to 7 days at room temperature make this test very attractive for daily routine.

Diagnosis of HE continues to be a major clinical problem. Patients may present with mild cognitive impairment. It is important to recognize these complications and their early stages because adequate treatment of the condition reduces morbidity and mortality.

To address these issues, we examined whether fecal calprotectin represented a useful diagnostic tool for liver cirrhosis and hepatic encephalopathy. This may help assessing HE severity which may be subjective when using clinical criteria alone.

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Conclusion

Fecal calprotectin concentration is found to be elevated in cirrhotic patients and correlated with the severity of liver disease as assessed by Child Turcotte Pugh score and MELD Na score. Fecal calprotectin is significantly elevated in hepatic encephalopathy patients compared to cirrhotic patients without hepatic encephalopathy. Higher values of fecal calprotectin concentration are found as the grades of hepatic encephalopathy increase. There is significant correlation between its values and grade 1, 2 and 3 of hepatic encephalopathy according to West Haven criteria. Fecal calprotectin can be used as a diagnostic marker for hepatic encephalopathy and may be of help in assessing severity of the disease.

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