

COMPARATIVE STUDY BETWEEN SPONTANEOUS FUNGAL PERITONITIS (SFP) AND SPONTANEOUS BACTERIAL PERITONITIS (SBP) IN PATIENTS WITH END STAGE LIVER DISEASE (ESLD)

By

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ABSTRACT

Background: Bacterial infections in end stage liver disease are common and associated with increased mortality. Although extensive research has elucidated the impact of bacterial infections as the major precipitating event leads to increased frequency of liver-related complications and mortality in these patients.

Objective: Evaluating the clinical and laboratory characters, risk factors, and outcome of fungal and bacterial peritonitis in cirrhotic patients with end stage liver disease (ESLD).

Patients and Methods: This cross sectional study enrolled 60 patients with liver cirrhosis and ascites. Fifty patients were culture-positive spontaneous peritonitis, including 12 with spontaneous fungal peritonitis (SFP), and 38 culture-positive spontaneous bacterial peritonitis (SBP), and 10 patients without clinical or laboratory evidence of peritonitis as a control group, during the study period, between 1st February 2018 and 30th May 2019. We compared the clinical, laboratory findings, Child–Pugh scores, model for end stage liver disease (MELD) score, risk factors, and mortality rates between patients with spontaneous peritonitis associated with fungal culture-positive ascites, and those with spontaneous peritonitis associated with bacterial culture-positive ascites.

Results: Patients of the current study were critically ill. The mean duration of ESLD in the studied patients was 4.75 ± 1.8 year, 57 patients (95.0%) were Child score C, and 3 patients (5%) were Child score B. The mean MELD score was 23.32 ± 5.49 . All patients with evidence of peritonitis were Child C, and mean MELD scores were 22.6 ± 5.7 and 26.1 ± 4.5 in SBP and SFP groups respectively. Jaundice, GI bleeding and hepatic encephalopathy were significantly for clinical presentation and predictors of mortality in SFP group compared to SBP and control group. Lower levels of hemoglobin (Hb), S. albumin and platelets count and increased level of total leukocytic count (TLC), international normalized ratio (INR), and bilirubin were significantly higher in SFP group compared to SBP and control group.

Duration of ESLD and hospital admission was significant in SFP compared to SBP and control group. HCC was evident in SFP group (66.7%) that was highly significant in comparison to SBP (28.9%), and control group (20%). Overall mortality rate among the studied groups was 45%, the mortality rate was significantly increased in SFP group (75%) compared to SBP (42.1%), and control group (20%). The mean MELD score significantly increased in non-survival group compared to survival group. The duration of ESLD significantly increased in non-survival group compared to survival group.

Conclusion: Prolonged and severe underlying liver disease with a high Child- Pugh or MELD score, renal impairment, HCC, and onset of severe sepsis have been reported as risk factors in increased mortality in

patients with SFP than in SBP. In SFP, mortality was associated not only with the severity of the underlying liver disease, but also with delay in diagnosis and initiation of antifungal therapy.

Keywords: fungal infection, liver cirrhosis, Spontaneous fungal peritonitis.

INTRODUCTION

Patients with liver cirrhosis (LC) are at a high risk of developing bacterial and fungal infections such as spontaneous bacterial peritonitis (SBP) or spontaneous fungal peritonitis (SFP), which can be life-threatening in this population. Various mechanisms predispose patients with LC to infections. Impairment of immune function has been well documented and is characterized by multiple immune deficiencies, involving not only local liver damage but also deficiencies in systemic innate and acquired immunity (*Albillos et al, 2014*). Increased gastrointestinal (GI) permeability and pathological bacterial translocation are considered key factors leading to increased infection susceptibility in LC (*Bellot et al, 2013*).

The clinical features and prognosis of SBP, the most common type of bacterial infectious disease affecting patients with, have been well documented. Furthermore, in bacterascites, a subtype of SBP, bacteria may be cleared naturally from ascitic fluid. Accordingly, the need for antibacterial therapies in such cases remains uncertain. In contrast, the features of SFP (or fungiascites) in patients with liver cirrhosis remain less well-known, and few studies have compared SFP and SBP or, more generally, spontaneous peritonitis caused by fungi (SFP or fungiascites) with those caused by bacteria (SBP or bacterascites) (*Shizuma, 2018*). To address this differences the study was conducted on 60 patients with Chronic liver disease to estimate Clinical characteristics, risk factors, and prognosis

of spontaneous fungal peritonitis (SFP) in comparison to spontaneous bacterial peritonitis (SBP) in patients with end stage liver disease (ESLD).

The aim of this work was to evaluate clinical and laboratory characters, risk factors, and outcome of fungal and bacterial peritonitis in cirrhotic patients with ESLD.

PATIENTS AND METHODS

For this study, we enrolled 60 patients with chronic liver disease admitted to the Hepato-Gastroenterology and Infectious diseases Departments, Al-Azhar University, and Al-Helal Hospital, Cairo, Egypt, from 1st February 2018 and 30th May 2019. Fifty patients were classified into 2 groups: SFP Group included 12 patients with positive culture for fungal peritonitis; SBP group included 38 patients with positive culture for bacterial peritonitis, in addition to control group which included 10 patients with end stage liver disease. All patients who met the diagnostic criteria of culture-positive spontaneous peritonitis were selected and included in this study. The diagnostic criteria of culture positive spontaneous peritonitis were as follows: Ascitic fluid polymorphonuclear leukocyte (PMN) count >250 cells/mm³, positive ascitic fluid culture and absence of intra-abdominal sources of infection or inflammation proven by abdominal imaging. Patient's sero-positive for human immunodeficiency virus (HIV) and patients undergoing continuous

ambulatory peritoneal dialysis were excluded from the study.

All patients were subjected to full history and clinical examination. Routine laboratory evaluation and imaging study were done. All patients were subjected to diagnostic paracentesis immediately after admission or upon presentation of signs and/or symptoms suggestive of peritonitis. Ten milliliters of ascetic fluid were collected at the bedside and inoculated into aerobic and anaerobic blood culture bottles. Bacterial culture was performed manually using MacConkey and blood agar plate. Fungal culture was performed in the same manner using Emmons Sabouraud dextrose agar (ESDA). The ascetic fluid PMN count was determined simultaneously, and empirical antibiotics were administered immediately after diagnostic paracentesis. The ascetic fluid PMN count and cultures were determined 48 h after the start of treatment for all SFP and SBP patients. The criteria used to consider the antibiotic therapy successful were negative results of ascites and blood cultures; and a sustained decrease in the ascitic PMN count to <50 % of the initial value 48 h after the initiation of antibiotics.

All patients involved in the current study were informed about the nature and details of the current work and a written consent was obtained for each one.

Statistical analysis:

The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

i. Descriptive statistics:

1. Mean, Standard deviation (\pm SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non-parametric numerical data.
2. Frequency and percentage of non-numerical data.

ii. Analytical statistics:

1. Student t test was used to assess the statistical significance of the difference between two study group means.
2. ANOVA test was used to assess the statistical significance of the difference between more than two study group means.
3. Post Hoc Test was used for comparisons of all possible pairs of group means.
4. Chi-Square test was used to examine the relationship between two qualitative variables.
5. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.
6. Logistic regression was useful in the prediction of the presence or absence of an outcome based on a set of independent variables. It is similar to a linear regression model but is suited when the dependent variable is qualitative (categorical).

RESULTS

The present study was conducted on 60 patients with chronic liver diseases admitted to the Tropical Medicine Department, Al-Azhar University, and Al-Helal Hospital Cairo, Egypt, from 1st February 2018 and 30th May 2019. Fifty patients were classified into 2 groups: SFP group included 12 patients with positive culture for fungal peritonitis, and SBP group included 38 patients with positive culture for bacterial peritonitis, in addition to control group which included 10

patients with end stage liver disease matched with the previous two groups with no evidence of peritonitis, which were admitted for different reasons.

Male gender was significantly higher in control group than SBP and SFP group, and female gender was not represented in the control group, and was significantly higher in SFP group. There was no significant difference regarding to age between the studied groups (**Table 1**).

Table (1): Comparison between the demographic data among the studied groups

Parameters		Control No. = 10		SBP No. = 38		SFP No. = 12		Fisher exact test
		N	%	N	%	N	%	p value
sex	Male	10	100.0%	29	76.3%	6	50.0%	0.028
	Female	0	0.0%	9	23.7%	6	50.0%	
Age (years)		Mean	SD	Mean	SD	Mean	SD	ANOVA
		54.6	11.2	57.7	6.7	58.5	7.5	

There was no significant difference between the studied groups regarding to the Child score, but there were significant differences regarding to overall mortality.

The highest mortality rate was among the SFP group 9/12 patients (75.0%) versus 16 (42.1%) and 2 (20.0%) in SBP and control group respectively (**Table 2**).

Table (2): Outcome of patient's post-treatment with anti-fungal therapy

Parameters		Control No. = 10		SBP No. = 38		SFP No. = 12		Test of sig.
		N	%	N	%	N	%	p value
Child score	B	2	20.0%	1	2.6%	0	0.0%	0.095
	C	8	80.0%	37	97.4%	12	100.0%	
Death	No	8	80.0%	22	57.9%	3	25.0%	0.030
	Yes	2	20.0%	16	42.1%	9	75.0%	
HCC	No	8	80.0%	27	71.1%	4	33.3%	0.041
	Yes	2	20.0%	11	28.9%	8	66.7%	

The mean meld score was significantly higher among the non survivors (27.6 ± 4.0) versus survivors (19.2 ± 3.3), the duration of ESLD per years was also

significantly higher in non survivors (5.7 ± 2) versus in survivors (4.1 ± 1.5) (**Table 3**).

Table (3): Comparison regarding to the mortality rate between SBP and SFP based on MELD score and duration of ESLD

Parameters	Groups	Survivors No. = 33		Non survivors No. = 27		p value
		Mean	SD	Mean	SD	
MELD		19.2	3.3	27.6	4.0	<0.001
Duration of ESLD (years)		4.1	1.5	5.7	2.0	0.004

There was no significant difference between the survivors and non survivors regarding to the Child score, but there were significant differences regarding the

mortality rate in patients with HCC (88.2%) (This table displays the odds ratios 17.25) **Table (4).**

Table (4) Logistic Regression analysis between survivors and non survivors regarding to Child score and presence of HCC

Parameters	Groups	Survivors		Non survivors		Fisher exact test	OR (95%CI)
		N	%	N	%	p value	
Child score	B	1	100.0%	0	0.0%	1.000	
	C	24	49.0%	25	51.0%		
HCC	No	23	69.7%	10	30.3%	<0.001	17.25 (3.31 - 89.97)
	Yes	2	11.8%	15	88.2%		

The mean value of leucocytic count, INR, blood urea and creatinine, potassium level and serum bilirubin was a significant

variable in prognosis of patients with peritonitis regarding to mortality rate **(Table 5).**

Table (5): Comparison regarding to the mortality rate between SBP and SFP based on laboratory findings

Parameters	Groups	Survivors		Non survivors		p value
		Mean	SD	Mean	SD	
Hb (gm/dL)		9.8	1.5	9.3	1.9	0.271
WBC (x10 ³ /mL)		9.0	4.3	13.3	7.9	0.022
PLT (x10 ³ /mL)		92.0	20.8	81.9	54.9	0.397
INR		1.5	0.2	1.9	0.5	0.002
Urea (mg/dL)		65.4	34.5	97.0	45.4	0.008
Creatinine (mg/dL)		1.4	0.5	2.6	1.1	<0.001
Na (mmol/L)		134.8	6.5	135.4	9.3	0.806
K (mmol/L)		4.2	0.5	4.6	0.6	0.008
ALT (IU/L)		25.0	9.6	31.0	8.1	0.763
AST (IU/L)		40.0	11.4	37.0	10.8	0.793
Albumin (gm/dL)		2.5	0.3	2.3	0.6	0.137
Total bilirubin (mg/dL)		2.0	0.6	2.8	1.0	0.001

There was a positive correlation between the occurrence of SFP and and

prolonged INR and long duration of ESLD $r = 0.6$ **(Fig. 1).**

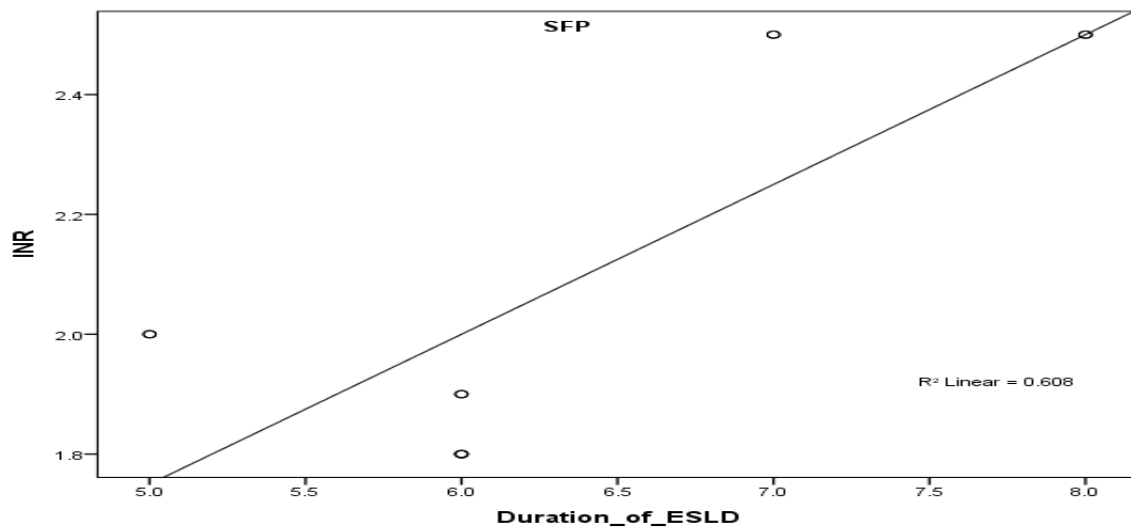


Figure (1): Correlation between the duration of ESLD, INR and occurrence of SFP

There was a positive correlation between the occurrence of SFP and increased MELD score $r = 0.3$ (Fig. 2).

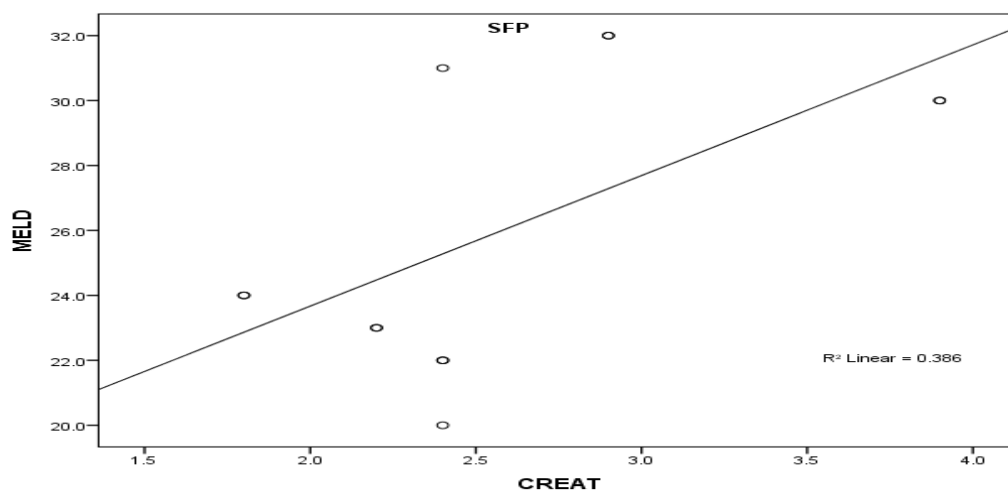


Figure (2): Correlation between MELD score and occurrence of SFP

DISCUSSION

The relationship between immune dysfunction and infection in cirrhosis, known as cirrhosis-associated immune dysfunction syndrome (CAIDS), has been investigated extensively. This syndrome is a multifactorial state of dysimmunoregulation, which is comprised of a reduction in serum bactericidal activity, opsonic activity, and complement

and fibronectin levels (Bonnell *et al.*, 2011).

The current work was one of few studies aiming to report the clinical character, risk factors, and prognosis of cirrhotic patients with fungal peritonitis compared to bacterial peritonitis.

Out of the total 50 cases of cirrhotic ascites, 75% were males and 25% were females. This high male to female ratio

was due to the criteria of selection of patients at the beginning of the study. The incidence of bacterial and fungal peritonitis in the current study was 63.3% and 20% respectively. That disagreed with *Hwang et al. (2014)* who reported the incidence of SFP was 3.6% in Korean tertiary care center and also with *Hassan et al. (2014)* who reported the incidence of SFP in Egyptian population was 4.3%, which may be due to the excessive use of prophylactic antibiotics in our selected patients.

Upper GI bleeding was evident in all patients with SFP and significantly increased compared to SBP and control group. This result agreed with *de Mattos et al. (2014)* who stated that the gastrointestinal bleeding is a predictor of incidence of SBP. Also, *Hassan et al. (2014)* stated that fungal translocation may be facilitated by upper gastrointestinal bleeding, which is also common in patients with advanced liver cirrhosis.

High serum bilirubin, s.creatinin and low serum albumin and hyponatremia was a significant risk factors among the studied groups the results is matched with the report of *Fernandez et al. (2016)* that the presence of low ascitic protein concentration (<1.5 g/dL) when combined with any of the following characteristics: Child-Pugh score ≥ 9 , serum bilirubin level ≥ 3 mg/dL, impaired renal function (creatinine ≥ 1.2 mg/dL or blood urea nitrogen level ≥ 25 mg/dL), or hyponatremia (≤ 130 mEq/L) are a recognized risk factors in spontaneous peritonitis (*Fernandez et al., 2016*).

Another recognized risk factor for spontaneous peritonitis in the current

study was higher child score among the studied group (95 %) were child C, and (5%) were child B. All SFP patients were Child C and 37/38 (97.4%) of SBP group were child C and 80% of control group were child C the finding that explain that the severity of liver impairment was associated with spontaneous bacterial and fungal peritonitis. These results agreed with *Fiore et al. (2016)* who reported that patients with cirrhosis are at an increased risk of fungal infections, and with *Hwang et al. (2014)* who analyzed the clinical characteristics and the prognosis of patients with spontaneous peritonitis, 3.6% of whom presented spontaneous fungal peritonitis. In those patients, the most frequent isolate was *Candida albicans*, followed by *Candida glabrata*, *Candida krusei*, *Cryptococcus spp.*, and *Aspergillus spp.*

High MELD score was an additional recognized risk factor in the occurrence of spontaneous bacterial and fungal peritonitis among the studied groups. The mean MELD score was significantly increased in SFP than SBP and control group. The result matched with the study of *Rashid et al. (2019)* who stated that patients and control SBP was 11%, 31%, 71%, and 93% at baseline MELD scores less than or equal to 10, from 11 to 20, from 21 to 30, and greater than 30, respectively. Compared with MELD score less than or equal to 10, patients with MELD scores were from 11 to 20, 21 to 30, and greater than 30 had six folds (95% CI) risk of SBP (*Rashid et al., 2019*).

High Child-Pugh or MELD scores increased the risk of SFP in patients with liver cirrhosis. A retrospective case-control study by *Gravito-Soares et al.*

(2017) found no significant difference in the Child-Pugh or MELD scores of patients with SFP and SBP.

The mean duration of ICU admission was significantly higher in patients with SFP compared to SBP and control group. This agreed with *Gravito-Soares et al. (2017)* who reported that the long duration of hospital admission is a risk factor for SFP occurrence.

The overall mortality in the current study was 45%. The highest mortality was among the SFP group than in SBP and control group (75%, 42.1% and 20% respectively). Predictors of mortality in SFP and SBP include severe underlying liver disease with a high Child-Pugh or MELD score, renal impairment such as HRS, and onset of severe sepsis. Our results were in concordance with that reported by *Bal et al. (2016)* who reported that the predictors of mortality in SBP was the same factors reported in our study. Other predictors of SFP and SBP mortality in the current study included prolonged admission in the IC unit, other comorbidities, GI bleeding, rapid deterioration of liver function, positive ascitic fluid fungal culture, elevated blood leukocyte level, and presence of HCC. *Tandon et al. (2011)* reported that peripheral blood leukocyte count $\geq 11 \times 10^9$ cells/L and MELD score ≥ 22 were independent predictors of 30-d mortality in patients with liver cirrhosis and SBP.

SFP mortality is estimated to be 56%-90% in multiple studies (*Hassan et al., 2013, Bremmer et al., 2015 and Gravito et al., 2017*), and 1-mo mortality may or may not be significantly higher than SBP mortality. *Hwang et al. (2014)* reported a high SFP mortality that was related to

unresponsiveness to initial empirical treatment for suspected SBP. The condition of nearly all the patients with SFP worsened after initial empirical treatment, and they died during the early stage of peritonitis regardless of undergoing antifungal treatment. Some patients with SFP improved after receiving the initial empirical treatment without any antifungal agents. Those patients may have had SBP and colonization by innocent fungi, as it was not possible to distinguish fungal colonization from true SFP in the clinical setting. SFP is usually diagnosed after the identification of fungi in cultures of ascitic fluid. The mortality was high because of delayed diagnosis, lack of clinical signs, lack of suspicion of SFP, and delay in treatment with antifungal therapy. Fungal resistance to empirical specific antifungal therapy together with delayed diagnosis and treatment is related to poor prognosis of SFP (*Gravito et al., 2017*).

The highest incidence of mortality in SFP group (75%) may also due to delayed diagnosis, lack of clinical signs specific to SFP, lack of suspicion and delay in treatment with antifungal therapy as that is reported by *Gravito et al. (2017)*.

CONCLUSION

SFP is not a rare complication in end stage liver disease. SFP is underrated and associated with increased mortality rate as compared to SBP. Physicians should be aware of SFP in patients with CHILD C liver cirrhosis, elevated MELD score, antibiotic pretreatment and nosocomial peritonitis. SFP is an important complication of cirrhosis that is associated with high rates of death. Hence, to improve the outcome in these patients,

clinicians should have a high index of suspicion for this lethal entity so that it may be detected early, and empiric antifungal treatment should be started, especially in high-risk patients, until appropriate treatment can be used.

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دراسة مقارنة بين الالتهاب البريتوني التلقائي الفطري والبكتيري في مرضي الكبد بالمرحلة المتقدمة

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خلفية البحث: الالتهابات البكتيرية التلقائية في مرضي الفشل الكبدي شائعة وترتبط بزيادة الوفيات. علي الرغم من أن الأبحاث المستفيضة أوضحت تأثير الالتهابات البكتيرية التلقائية حيث أنها تؤدي الي زيادة تواتر المضاعفات و الوفيات المرتبطة بالكبد لدي هؤلاء المرضي، الا أنه لا يُعرف سوى القليل عن دور الالتهابات الفطرية التلقائية.

الهدف من البحث: تقييم التشخيصات السريرية والمختبرية وعوامل الخطورة ونتائج الالتهاب البريتوني الفطري والبكتيري التلقائي في مرضي الفشل الكبدي. ولكي يتم تحقيق هذا الهدف قمنا بمتابعة عدد ٦٠ مريض من مرضي الكبد في المراحل المتقدمة و المصابين باستسقاء في البطن في الفترة من فبراير عام ألفان و ثمانية عشر إلي مايو عام ألفان و تسعة عشر في قسم الكبد و الجهاز الهضمي بكلية الطب جامعة الأزهر ومستشفى الهلال الأحمر.

المرضي و طرق البحث: هذه دراسة مقطعية وصفية إحتوت علي خمسون مريضا تم تشخيصهم بالالتهاب البريتوني التلقائي بناء علي مزرعة من السائل البريتوني، منهم إثني عشر مصابون بالالتهاب البريتوني الفطري التلقائي، و ثمانية و ثلاثون مصابون بالالتهاب البريتوني البكتيري التلقائي ، بالاضافة إلي عشرة مرضي لا يبدو عليهم أي أعراض للالتهاب البريتوني التلقائي سواء سريرية او معملية كمجموعة حاكمة. وقد تم تجميع البيانات التي تخص الحالات مثل السن ومحل الإقامة ووقت تشخيص الفشل الكبدي، وأيضا تم تجميع المعلومات عن تحاليل المرضي مثل صورة الدم ووظائف الكبد والكلبي، وعمل الموجات الصوتية ومزارع من السائل البريتوني. وقد تمت مقارنة النتائج بين المجموعتين اللتان تم تشخيصهم بالالتهاب البريتوني التلقائي سواء فطري او بكتيري.

نتائج البحث: أوضحت النتائج أن مدة الفشل الكبدي في الدراسة كانت في المتوسط ٤,٨ عام، وقد حصل ٥٧ مريض علي التقييم ج في مؤشر تصنيف تشيلد و حصل ٣ مرضي علي التقييم ب في نفس المؤشر. وكان عدد نقاط مؤشر ميلد في المتوسط ٢٣,٣٢ نقطة، وكان عدد نقاط المؤشر أعلي في حالة الالتهاب الفطري. وقد كان إرتفاع نسبة الصفراء والغيبوبة الكبدية والنزيف من الجهاز الهضمي أفضل مؤشر اكلينيكي للالتهاب البريتوني الفطري، وكذلك أفضل مؤشر لنسبة الوفاة ، وذلك بالمقارنة بمجموعة التحكم ومجموعة الالتهاب البريتوني البكتيري.

وكان انخفاض نسبة الهيموجلوبين و قلة عدد الصفائح و ارتفاع نسبة عدد الكرات البيضاء و انخفاض نسبة الالبومين في الدم مؤشرا قويا علي وجود الالتهاب البريتوني الفطري التلقائي. كما كان طول مدة الفشل الكبدي و الإقامة بالمستشفى أعلي في الالتهاب البريتوني الفطري التلقائي.

وكانت نسبة الاورام الكبدية أعلي في الالتهاب الفطري التلقائي ٨ مرضي بنسبة ٦٦,٧% منها في الالتهاب البكتيري ١١ مريض بنسبة ٢٨,٩%. و كانت نسبة الوفيات أعلي في الالتهاب الفطري التلقائي (٩ حالات بنسبة ٧٥% بالمقارنة بالالتهاب البكتيري التلقائي ١٦ حالة ٤٢,١%) و مجموعة التحكم حالتين (٢٠%). و كانت عدد نقاط مؤشر ميلد أعلي في الحالات المتوفاة بنسبة ٢٧,٦ في المتوسط مقارنة ب ١٩,٢ في المتوسط بالنسبة للحالات التي كانت على قيد الحياة ، كما كانت مدة الفشل الكبدي أطول في الحالات المتوفاة ٥,٧ سنة في المتوسط مقابل ٤,١ سنة للباقي.

الاستنتاج: طول مدة ودرجة المرض الكبدي بالاضافة الي ارتفاع نقاط مؤشري تصنيف تشيلد و مؤشر ميلد ووجود ضعف في وظائف الكلي ووجود اورام كبدية سرطانية هي كلها عوامل خطرة تزيد من نسبة الوفيات في مرضي الالتهاب البريتوني الفطري التلقائي أكثر منها في الالتهاب البريتوني البكتيري التلقائي . وليست هذه النقاط هي المؤثرة فقط في نسبة الوفيات في الالتهاب الفطري، ولكن ايضا التأخر في بدء العلاج بمضادات الفطريات، و تأخر التشخيص يؤدي الي نتائج أسوأ.