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SIGNIFICANCE OF ASCITIC FLUID FERRITIN & LDH IN DIFFERENTIATING MALIGNANCY RELATED ASCITES FROM BENIGN ASCITES

Gastroenterology

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ABSTRACT

Introduction: Simple test(s) on ascitic fluid or serum which can help differentiate between Malignancy and Non-Malignancy Related ascites is a challenge that is not always met satisfactorily.

Aim: The purpose of this study is to assess the usefulness of Ascitic Fluid Ferritin and Ascitic Fluid Lactate Dehydrogenase (LDH) in differential diagnosis of Ascites.

Methodology: This prospective study included 80 patients with Ascites, admitted to Medical Gastroenterology Department of Madras Medical College, from January 2018 to January 2019. The patients were divided into two groups – Malignant ascites (n=30) and Non-malignant Ascites (n=50). The modalities for selecting malignant group were either cytology/ peritoneal biopsy positive cases . Serum and ascitic fluid ferritin and LDH were analyzed in all cases. Bacterial peritonitis was excluded in all these patients. Data were entered in Microsoft Excel and analyzed using IBM SPSSS (Ver. 20.0)

Results: Mean value of Ascitic Fluid Ferritin were significantly higher in Malignancy group than Non-malignancy group (1108.3±1236.2 vs 117.5±115.6 ng/ml) (p value 0.001). In the malignant group, mean value of LDH was 659.9±304.5 IU/ml, and in benign group it was 93.4±64.9 IU/ml (p value 0.001). Ferritin at a cut off value of ≥350 has a sensitivity of 90% and specificity of 92% and LDH at a cut off value of ≥150 has 90% sensitivity and specificity for differentiating malignant and benign ascites.

Conclusion: Ascitic fluid Ferritin and LDH, which are simple, easily available and cost-effective markers, can be used to differentiate Malignancy Related Ascites from Non-malignant Ascites.

KEYWORDS

ascites; ferritin; lactate dehydrogenase; malignant

INTRODUCTION

Usually, Malignant ascites is caused by lung, breast, ovarian, endometrial, colorectal, pancreatic, hepatobiliary, and primary peritoneal carcinomas; and it accounts for about 10% of all cases of ascites [1]. For further diagnostic and therapeutic procedures, it is important to differentiate between malignancy-related ascites (MRA) and non-malignant ascites (NMA). Due to poor sensitivity, Cytology is not a good screening tool for malignant ascites. Furthermore, reactive mesothelial cells in the ascitic fluid are lookalikes of malignant cells. Hence, it is difficult to distinguish between the two, based on morphology alone [2]. So, simple tests on ascitic fluid or serum, which can be used to differentiate between malignancy-related ascites (MRA) and non-malignant ascites (NMA) will be a blessing in solving this diagnostic predicament.

Even though Lactate dehydrogenase (LDH) is a cytoplasmic marker, it has also been used as a tumor marker. The presence of malignant cells in ascitic fluid is heralded by a raise of LDH in ascitic fluid [3]. Ferritin is an iron storage protein with a molecular weight of about 450,000 Dalton. It is mainly found in reticuloendothelial and liver system. Normally, in the serum and other body fluids, traces of ferritin are present. In healthy adults, the concentration of ferritin in serum is positively correlated with the iron stores in the body [4]. Increased serum concentrations of ferritin have been found in patients with iron overload, severe liver disease, especially in alcoholic liver disease and viral or drug induced hepatic necrosis [5]. Ferritin possibly behaves as an acute phase protein [6] in the inflammatory response. For certain malignancies like lymphoma, acute leukemia, multiple myeloma, breast, testicular, head and neck, hepatocellular, lung, thyroid, colon and pancreatic cancers, Ferritin is also used as a tumor marker [7]. The increased concentrations of ferritin associated with malignant ascites can be due to several mechanisms like increased synthesis of ferritin associated with inflammation, increased secretion of ferritin by malignant cells and hepatocellular necrosis caused by liver metastases [8].

Ascitic fluid ferritin and LDH levels has been proposed and investigated as a marker of malignant ascites previously with conflicting results. In the current study, we further examine the potential usefulness of these markers in ascitic fluids for the differential diagnosis of malignant and cirrhotic ascites.

AIM

The purpose of this study is to assess the usefulness of ascitic Ferritin and LDH, which are less expensive and easily available markers, in diagnosis of Malignant and Non-malignant Ascites.

METHODOLOGY

This prospective study included 80 patients with Ascites admitted to Medical Gastroenterology Department from January 2018 to January 2019. The patients were divided into two groups – Malignant (n=30) and Non-malignant (n=50). The modalities for selecting malignant group were either cytology/ peritoneal biopsy positive cases or cases with liver secondaries. Complete Blood Count, LFT, Ascitic Fluid Analysis including Total Protein, Albumin, Ferritin, LDH, culture & sensitivity, Total and differential counts and Cytology were done in all patients. USG abdomen, upper GI endoscopy, CECT abdomen, ADA, FNAC of peritoneal nodules and liver biopsy were performed in selected cases where it was indicated. Bacterial peritonitis was excluded in all these patients. Ferritin levels were measured using Automated Bidirectionally Interfaced Chemiluminescent Immuno Assay and LDH were measured using commercial kits.

Statistical Analysis:

Data were entered in Microsoft Excel and analyzed using IBM SPSS Software Version 20.0. Percentage Analysis was used for categorical variables (Gender, Etiology). Mean with Standard Deviation or Median with Inter-quartile range (IQR) were used for continuous variables (Age, Ascitic Fluid Protein, Ferritin and LDH) depends on the normal distribution. Comparison of Parametric Data and Non-Parametric data between two groups were done by using Student's t test (Unpaired t test). Comparison of categorical variable between the groups (gender with type of Ascites) was done by using Chi-Square test. Discriminatory performance of variables was determined by area under the receiver operating characteristic (ROC) curve, and best cut-off values were calculated based on operating characteristic (ROC) curve, and best cut-off values were calculated based on the High Sensitivity and Specificity. A p value of <0.05 was considered statistical significance with 95% Confidence Interval.

RESULTS

Our study included 80 cases - 50 cases with non-malignant ascites

cases and 30 with malignant ascites.

Table 1: Demographic Analysis

Demographic Details	Malignant Ascites		Benign Ascites		t Test	P value
	Mean	SD	Mean	SD		
Age	56.7	10.5	49.2	11.9	t value= 2.877	0.005*

The mean age of the patients in malignant ascites group was 56.7±10.5 years and in benign ascites group was 49.2±11.9 years.

Table 2: Gender-wise Analysis

Gender	Malignant Ascites N (%)	Benign Ascites N (%)	Total N (%)	Chi-square test (df)	P value
Male	12 (40)	41 (82)	53 (66.3)	14.8 (1)	<0.001*
Female	18 (60)	9 (18)	27 (33.8)		
Total	30 (100)	50 (100)	80 (100)		

Among 80 cases, 53 (66.3%) were males and 27 (33.8%) were females.

Table 3: Distribution of Ascites based on Etiology:

Etiology	Number	Percentage
Cirrhosis	46	57.5
TB Ascites	2	2.5
Cardiac Ascites	1	1.3
Pancreatic Ascites	1	1.3
CA Ovary	12	15
CA Stomach	7	8.8
CA Bladder	2	2.5
CA Gall Bladder	2	2.5
CA Colon	2	2.5
CA Pancreas	1	1.3
CA Prostate	1	1.3
HCC	1	1.3
Cholangio Carcinoma	1	1.3
Pancreatic NET	1	1.3
Total	80	100

Out of 50 cases in benign group, 46 cases (57.5%) were having cirrhotic ascites, 2 cases (2.5%) with TB ascites and remaining ones with cardiac and pancreatic ascites (1.3%). In the malignancy group, out of 30 cases, 12 cases were carcinoma of ovary.

Table 4: Ascitic fluid Protein Analysis

Variable	Malignant Ascites		Benign Ascites		t Test	P value
	Mean	SD	Mean	SD		
Ascitic fluid Protein (g/dl)	3.73	1.3	1.5	0.8	0.57	**0.001***

For the malignancy group mean value of protein is 3.73±1.3 vs 1.5±0.8 in benign group. Student t test is applied, p value is 0.001.

Table 5: Serum-ascites Albumin Gradient (SAAG) Analysis

Variable	Malignant Ascites		Benign Ascites		t Test	P value
	Mean	SD	Mean	SD		
SAAG (g/dl)	0.7	0.22	1.88	0.52	-14.1	**0.001***

The mean value of SAAG was higher in non-malignant group 1.88+-0.52 vs 0.7+-0.22 in malignant group. Students t test is applied, and p value is 0.001.

ASCITIC FLUID FERRITIN

Table 6: Ascitic Fluid Ferritin Analysis

Variable	Malignant Ascites		Benign Ascites		t Test	P value
	Mean	SD	Mean	SD		
Ascitic fluid ferritin (ng/ml)	1108.3	1236.2	117.5	115.6	-5.65	**0.001***

Table 7: Sensitivity & Specificity of Ferritin

Variable	AUC	Level ≥350	
		Sensitivity	Specificity
Ascitic Fluid Ferritin	0.917	90%	92%

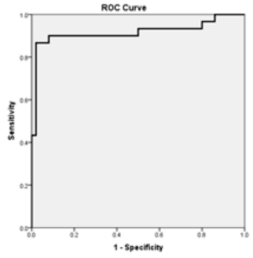

Fig. 1: Receiver operating characteristics (ROC curve) of Ascitic Fluid Ferritin

Mean value of Ascitic Fluid ferritin were significantly higher in Malignancy group than Non-malignancy group (1108±1236.4 vs 117.5±115 ng/l) P<0.001. Ascitic Fluid ferritin at a cut-off value of ≥350 ng/ml (sensitivity 90% and specificity 92%) differentiates Malignant & Benign group of Ascites. In both tubercular and pancreatic ascites cases also ascitic fluid ferritin was below 350ng/ml. Mean serum ferritin values was also high in malignant ascites 412ng/ml vs 168 ng/ml in benign ascites. No correlation was found between concentration of ferritin in ascites and in serum.

ASCITIC FLUID LDH

Table 8: Ascitic Fluid LDH Analysis

Variable	Malignant Ascites		Benign Ascites		t Test	P value
	Mean	SD	Mean	SD		
Ascitic fluid LDH (IU/L)	659.9	304.5	93.4	64.9	-12.7	**0.001***

Table 9: Sensitivity & Specificity of LDH

Variable	AUC	Level ≥150	
		Sensitivity	Specificity
Ascitic Fluid LDH	0.969	90%	90%

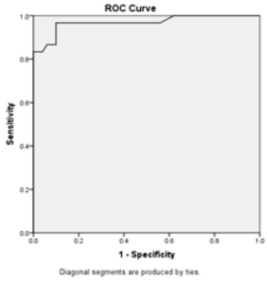

Fig. 2: Receiver operating characteristics (ROC curve) of Ascitic Fluid LDH

Mean value of Ascitic Fluid LDH in Malignancy related Ascites group was 659.9±304.5 vs 93.4±64.9 in Non-malignant group (P<0.001). Ascitic fluid LDH cut off level ≥150 has good sensitivity and specificity (90% & 90% respectively) in differentiating malignant and benign causes of ascites. Serum LDH levels were also high in malignant group 560 IU/l vs 326 IU/l in benign group.

DISCUSSION

The search for novel biochemical markers in the serum and/or ascitic fluid for differentiating malignant and non-malignant ascites is still under investigation. To identify malignant effusions, various biochemical markers have been employed in the past, because it is difficult to demonstrate malignant cells in effusions [9, 10]. A simple, quick and reliable test on ascitic fluid is essential, if the diagnosis is not obvious from the clinical presentation.

In exudative effusions of malignant and non-malignant natures, high

levels of ferritin are being observed [11, 12]. However, there are some opposing opinions on the use of effusion ferritin level in the discrimination of malignant and non-malignant exudates. While certain researchers claim that it can be a useful aid in the differential diagnosis of malignant and non-malignant exudates; some others opine that there was no difference between malignant and non-malignant effusion's ferritin level [12, 13]. But our study showed a significant difference in ascitic fluid ferritin and serum ferritin levels between both groups.

Several mechanisms may be responsible for the increased concentrations of ferritin associated with malignant disease. These include the increased synthesis of ferritin associated with inflammation, increased secretion of ferritin by malignant cells [14] and hepatocellular necrosis caused by liver metastases. The source of ferritin in ascites is unknown. Many of the proteins encountered in effusions are derived from the plasma. The concentration of such proteins in effusions depends primarily on the degree of membrane permeability and molecular size. Although increased serum ferritin levels often associated with malignant disease, it is unlikely that the origin of ferritin in ascites is serum ferritin. Firstly, no correlation has been found in previous studies between concentration of ferritin in ascites and in serum. Local ferritin concentrations exceeding many times those found in the serum cannot be assumed to originate from the circulation [15]. In our study also, ascitic fluid ferritin levels were very much higher than serum ferritin levels in malignant ascites. Secondly, because of its large size, it is unlikely that the ferritin molecule may pass through peritoneal membrane to any significant extent. By exclusion the main source of ferritin in ascitic fluid seems to be in situ production. Whether the direct source of ascitic ferritin is increased secretion by macrophages, disintegration of malignant or inflammatory cells, or specific production by malignant tissues is at present unknown. In our study, very high levels of ferritin were observed in cases of ca ovary. However, significance of this is not known. In view of the overlap between ferritin concentrations obtained in non-malignant inflammatory ascites and malignant ascites, the most likely source of ferritin in malignant ascites were inflammatory cells stimulated by the presence of tumor [13].

In our study we included 80 patients. The patients were divided in to malignant ascites (n=30) and benign ascites group (n=50). The mean ferritin level was found to be 1108.3ng/ml in malignant ascites group and 117.5ng/ml in benign group with a p value of 0.001. Ascitic fluid ferritin at a cut-off value of ≥350ng/ml, the sensitivity was 90% and specificity was 92%.

In Lt Col Mithu Banerjee et al study [16], Ascitic fluid ferritin values in malignant group were 475 (± 282) ng/mL and 52 (± 42) ng/mL in benign ascites group. The difference was found to be statistically highly significant. At a cut-off value of 95 ng/mL, the sensitivity and specificity were 100% and 63%. In our study, sensitivity was lower (90%) as cut off value was higher (95ng/ml vs 350 ng /ml).

In M.Cemil SAVAŞ et al study [15], the diagnostic usefulness of ferritin measurements in cirrhotic and malignant ascites has been evaluated in 61 patients. In 22 patients with malignant disease, median ferritin concentration was 833 ng/mi in the ascitic fluid. In 39 patients with ascites from liver cirrhosis corresponding value was 108 ng/ml. There was statistically significant difference between these values ($p<0.01$). Discriminative value of ferritin levels (>375 ng/ml) in malignant and cirrhotic ascites were calculated. Diagnostic specificity and diagnostic sensitivity were 81.8%, 89.7% respectively. Lactate Dehydrogenase is widely distributed in many body tissues. It is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD+ as a hydrogen acceptor. Patients with malignant diseases show increased LDH activity in serum. up to 70% of patients with liver metastases and 20% to 60% of patients with other non-hepatic metastases have elevated total LDH activity. The ascitic LDH levels of the malignant group were significantly higher than those of non-malignant group in previous reported studies.

In our study, mean ascitic fluid LDH in malignant group was 659 IU/l vs 93 IU/L in benign group with a p value of 0.001. The sensitivity and specificity were 90% at a cut of level of ≥150 IU/L. In exudative ascites other than malignancy like in pancreatic and tubercular Ascites cases also LDH values were not more than 150 IU/l. Serum LDH levels were also high in malignant group 560 IU/l vs 326 IU/l in benign group.

E. E. L.Ekpe et al [18] also reported that Higher levels of ascitic LDH were seen in the malignant group (900.67±918.45 IU/l) when compared to the non-malignant group (199.29±194.53 IU/l). This was statistically significant ($P<0.05$). The diagnostic accuracy of LDH was 90.7%. Boyer et al. [17] have published a mean ascitic fluid LDH of 913±228 IU/L in malignant ascites. in Lt Col Mithu Banerjee et al study [16], the ascitic fluid LDH at a cut-off of 422 U/L had a sensitivity of 74% and a specificity of 60% in differentiating malignant and benign ascites group.

CONCLUSION

The measurement of ferritin and LDH levels may be a useful tool in the differential diagnosis of malignant ascites from benign ascites. Thus, if all ascitic fluid specimens are screened first with ferritin and LDH and if they are very high patient requires to be worked up thoroughly for malignancy. Hence, these parameters being simple and cost effective, can be widely used to differentiate non-malignant and malignant ascites, even in small centers with limited diagnostic facilities.

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