Biomarkers of malignant ascites—a myth or reality

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ABSTRACT

BACKGROUND

Ascitic fluid aspirate cytology, although reasonably specific is not a good screening tool for malignant ascites due to poor sensitivity. Simple test(s) on ascitic fluid or serum which can help differentiate between benign and malignant causes of ascites will be a boon. Ascitic fluid lactate dehydrogenase, cholesterol, and ferritin are the candidate markers evaluated in this study.

METHODS

Ascitic fluid cytology was done on 30 patients of malignant ascites. The modalities used for diagnosing malignant ascites were positive peritoneal biopsy or CT scan evidence of hepatic metastases. Ascitic fluid biochemistry was done in all these 30 patients as well as 30 cases of non-malignant ascites. The parameters analysed were cholesterol, lactate dehydrogenase, and ferritin. The biochemical parameters were estimated in serum as well.

RESULTS

Cytology had a sensitivity of 40% for the diagnosis of malignant ascites. Ascitic fluid cholesterol, lactate dehydrogenase, and ferritin had sensitivities of 70%, 74%, and 100%, respectively. Serum cholesterol, lactate dehydrogenase, and ferritin had sensitivities of 57%, and 91%, respectively.

CONCLUSION

Hence, these biochemical markers in ascitic fluid as well as serum can be good screening tools for the diagnosis of malignant ascites.

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Key Words: ascites; cholesterol; ferritin; lactate dehydrogenase; malignant

INTRODUCTION

Detection of malignant cells on cytology of ascitic fluid aspirates has been the cornerstone for diagnosis of malignant ascites. However, cytology is not a good screening tool for malignant ascites as it has a poor sensitivity. In addition, reactive mesothelial cells in the ascitic fluid are mimics of malignant cells thus

based on morphology alone, it is difficult to distinguish between the two.¹ Simple tests which can be done either on ascitic fluid or serum which can help differentiate between benign and malignant causes of ascites will be a boon in solving this diagnostic dilemma. In this study, we evaluated cholesterol, lactate dehydrogenase (LDH), and ferritin in the ascitic fluid as well as serum as markers of malignant ascites.

Lactate dehydrogenase is a cytoplasmic marker and has also been used as a tumour marker. The presence of malignant cells in ascitic fluid is heralded by a raise of LDH in ascitic fluid.² Cholesterol is a component of the cell membrane. When the fragile malignant cells break down in ascitic fluid, cholesterol from the cell membrane is released into the ascitic fluid.^{3,4} Ferritin is an acute phase reactant and it has also been utilised as a tumour marker for certain malignancies like lung, liver, breast and haematolymphoid malignancies.⁵

The study is designed to calculate the sensitivity of cytology and compare this with sensitivities and specificities of cholesterol, lactate dehydrogenase and ferritin levels in ascitic fluid as well as serum for the diagnosis of malignant ascites.

MATERIALS AND METHODS

Sixty patients of ascites attending gastrointestinal and MDTC OPDs were included in the study. Thirty of these patients, 16 females and 14 males, had malignant ascites due to either a peritoneal carcinomatosis or hepatic metastasis (Group I).

The modalities for the diagnosis of malignancy were either peritoneal biopsy positive for peritoneal carcinomatosis or liver secondaries proven on CT scan. Remaining 30, 20 men and 10 women, had ascites due to non-malignant causes (Group II). Primary hepatic malignancy was ruled out in all these patients by liver biopsy/ultrasonography/CT scan.

Abdominal Paracentesis

Ascitic tap was done in all patients within 24 hours of admission, preferably before any intervention was done, using a 20 gauge needle with strict aseptic precautions. Five of the 60 patients were on diuretic therapy before admission to the hospital. However, LDH, cholesterol, and ferritin are not likely to be affected with diuretic therapy.

Cytological Examination of Ascitic Fluid

The ascitic fluid of all patients in group I was subjected to cytological examination. Leishman and Papanicolaou stained cytopsin sediments were examined by two independent cytopathologists. The slides were reported as one of the following

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three categories: (a) no evidence of malignancy, (b) suspicious for malignancy or (c) malignant. For calculating sensitivity of cytology category (c) was taken a positive, categories (a) and (b) were taken as negative.

Biochemical Analysis of Ascitic Fluid

Cholesterol and LDH were estimated in serum and ascitic fluid using commercial kits manufactured by Autopak by CHOD PAP and UV kinetic methods, respectively on the day of sample collection. The linearity of LDH was 1200 U/L. The linearity of cholesterol was 500 mg/dL.

Ferritin was estimated using ELISA kit manufactured by Calbiotech Ltd. All samples were run in duplicates. Samples were stored at -20°C for a maximum of 30 days before analysis. ELISA was performed using monoclonal biotinylated antibodies immobilised on the surface of a microplate, the wells of which were treated with streptavidin.

Six calibrators were used with concentrations of 0, 10, 50, 150, 400, and 800 ng/mL. The sensitivity of the kit was 1.0 ng/mL. The intra-assay precision was 3.1% and inter-assay precision was 5.5%. The normal range of serum ferritin in males is 16–220 ng/mL and that in females is 10–124 ng/mL. The normal for cholesterol was taken as 90–200 mg/dL and LDH as 200–400 U/L at 37°C .

Statistical Methods

Unpaired *t* test was done to test statistical significance. The ideal cut-offs for all the analytes were established after generating the receiver operating characteristics (ROC) using SPSS 10.0.1. A note was made of the sensitivities and specificities at the ideal cut-offs. The combined sensitivities and specificities of the various analytes and cytology were calculated using the EPI INFO package.

RESULTS

Distribution of Patients

The distribution of patients in the malignant group (group I, $n=30$) according to the type of cancer was carcinoma ovary 8, carcinoma gall bladder 5, carcinoma colon 3, carcinoma stomach 3, carcinoma pancreas 1, hepatic metastasis with unknown primary 4, hepatocellular carcinoma 1, breast carcinoma 2, lung carcinoma 1, and MALToma small intestine 2. The distribution of patients in the non-malignant group (group II, $n=30$) was chronic HBV and HCV 20, alcoholic liver disease 3, ESRD 6, and congestive cardiac failure 1.

Biochemical Analysis in Ascitic Fluid and Serum

The mean and the SD of the results obtained of the various biochemical parameters estimated in ascitic fluid as well as serum in both group I, II is given in Table 1. Ideal cut-offs of the analytes were ascertained after plotting the ROC as illustrated in (Figures 1 and 2). The area under curve; ideal cut-offs; and the sensitivity and specificity at the respective cut-offs are given in Table 2.

Cytological Examination of Ascitic Fluid

The results of the cytological examination in ascitic fluid were, 12 cases positive for malignancy, 16 negative for malignancy, and two suspicious malignancy. For calculation of sensitivity, cases reported as “suspicious for malignancy” were taken as negative. The concordance rate between the two cytopathologists was 95%. One case, which was that of a 64-year-old man, a case of Jejunal MALToma had discordant ratings by the two cytopathologists. However, since one of the cytopathologists labelled the sample as malignant it was taken as positive. The sensitivity of cytology was 40%.

Comparative Analysis of Diagnostic Value of Cytology and Biochemical Analytes

Diagnostic value of cytology, ascitic fluid LDH, cholesterol, ferritin and their combined use in distinguishing between patients of malignant and benign ascites is given in Table 3.

DISCUSSION

Discrimination of malignant from benign causes of ascites is of paramount importance in the differential diagnosis of ascites because the therapy and management of the two groups is radically different. Castaldo et al⁶ have cited a sensitivity of 40–60% for cytology in their article. In our study, the sensitivity calculated was 40%. Lack of sensitivity may be due to the low number of neoplastic cells present in some ascitic samples.^{7–9} False-negative results can be caused by the difficulty in distinguishing between neoplastic and atypical inflammatory cells/mesothelial cells.^{7,9}

In our study, ascitic fluid LDH at a cut-off of 422 U/L had a sensitivity of 74% and a specificity of 60%. Ascitic fluid LDH therefore is a better index for screening of malignancy as a cause for ascites compared to cytology which has a sensitivity of 40% only. In our study, of the two cases with suspicious cytology one had an ascitic fluid LDH of 614 U/L. This case could therefore have been picked up by estimating ascitic fluid LDH. Boyer et al¹⁰ have published a mean ascitic fluid LDH of 913 ± 228 U/L in the malignant group.

In our study, the mean ascitic fluid cholesterol in group I, II were $95 (\pm 58)$ mg/dL and $58 (\pm 40)$ mg/dL, respectively. The

Table 1 Biochemical analysis of ascitic fluid and serum in group I, II.

Analyte	Group I Mean (SD)	Group II Mean (SD)	P value
Ascitic fluid (AF) LDH (U/L at 37°C)	508 (146)	415 (219)	0.038
AF cholesterol (mg/dL)	95 (58)	58 (40)	0.861
AF ferritin (ng/mL)	475 (282)	52 (42)	<0.001
Serum LDH	554 (171)	442 (201)	0.637
Serum cholesterol	157 (41)	184 (49)	0.895
Serum ferritin	511 (194)	157 (58)	<0.001

Figure 1 Receiver operating characteristics (ROC curve) of analytes in ascitic fluid; (A) ascitic fluid LDH, (B) ascitic fluid cholesterol, (C) ascitic fluid ferritin.

difference was not found to be statistically significant. At a cut-off of 67 mg/dL, the sensitivity was 70% and specificity was 60%. This parameter could therefore be used as an adjunct with cytology for screening for malignant ascites which would enhance the sensitivity of cytology alone by 30%. The two suspicious cases on cytology had cholesterol levels of 108 mg/dL and 88 mg/dL. One of these cases could have been picked up on estimating cholesterol in the ascitic fluid. Prieto et al have reported ascitic fluid mean cholesterol of 176.85 ± 46.05 mg/dL in the group with malignant ascites and 140.79 ± 44.99 mg/dL in the group without malignancy.¹¹ Our values are considerably lower than these values in both groups which can be attributed to a lower concentration of serum cholesterol in the Indian population compared to the Western population. Cholesterol is increased in malignancy due to tumour involvement of the serosal cavity. It may enter the cavity from the interstitial space because of obstructed lymph vessels or may be related to the increased permeability of the carcinomatous serous membrane.

Ascitic fluid ferritin values in group I, II were $475 (\pm 282)$ ng/mL and $52 (\pm 42)$ ng/mL. The difference was found to be statistically highly significant. At a cut-off of 95 ng/mL the sensitivity and

specificity was 100% and 63%. Ascitic fluid ferritin should thus be used as a screening tool for malignancy in ascitic fluid. Kountouras et al¹² compared the ascitic fluid ferritin concentrations with serum-ascites albumin gradient (SAAG), in their diagnostic ability for detection of malignancy in 60 patients with ascites: 29 with chronic liver disease alone and 31 patients with various neoplasms. Analysis of his data confirms that ascitic ferritin is a more accurate indicator of malignant ascites than SAAG. This new parameter is particularly helpful in distinguishing malignant ascites associated with hepatocellular carcinoma and/or metastatic liver disease from non-malignant ascites due to chronic liver disease alone. Our data show that malignant ascites, both due to peritoneal carcinomatosis as well as hepatic metastasis, was picked up by ascitic fluid ferritin. Both the cases of suspicious cytology had ascitic fluid ferritin levels of 182 and 190 ng/mL which could have been picked up by estimating ferritin in ascitic fluid. Thus, if all ascitic fluid specimens are screened first with ferritin it will significantly reduce the workload. Samples that turn out positive should undergo cytology as it has a high specificity unlike ascitic fluid ferritin which has a lower specificity of 63%.

Figure 2 Receiver operating characteristics (ROC curve) of analytes in serum; (A) serum LDH, (B) serum cholesterol, (C) serum ferritin.

Table 2 Ideal cut-offs of analytes in ascitic fluid and serum and sensitivities and specificities at the ideal cut-offs.

Analyte	Ideal cut-off	True positives	False positives	Sensitivity % (95% CI)	Specificity % (95% CI)	AUC (area under curve)
AF LDH	422	22	12	74 (54.1–87.7)	60 (40.61–77.3)	0.685
AF cholesterol	67	21	12	70 (50.6–85.2)	60 (40.6–77.3)	0.654
AF ferritin	95	30	11	100 (85.9–100)	63 (43.8–80.1)	0.897
Serum LDH	474	17	14	57 (37.4–74.5)	53 (34.3–71.6)	0.638
Serum cholesterol	164	17	20	57 (37.4–74.5)	33 (17.3–52.8)	0.371
Serum ferritin	266	27	06	91 (73.4–97.7)	80 (61.4–92.2)	0.838

The serum ferritin in group I, II were 511 (± 194) ng/mL and 157 (± 58) ng/mL, respectively, which was also statistically highly significant. The significant raise in group I could be attributed to the malignancy as well as the acute phase reactant property of ferritin. This group also had patients with ovarian carcinomas with cachexia who had concomitant anaemia of chronic disease which could be a reason for the raised ferritin levels in this group. The serum ferritin level at a cut-off of 266 ng/mL had a sensitivity and specificity of 91% and 80%, respectively.

Both the cases of suspicious cytology had serum ferritin levels of 638 and 567 ng/mL which could have been picked up by estimating ferritin in serum. Ascitic fluid cholesterol, LDH and ferritin when done as a panel have a very high specificity of 93% with a sensitivity of 61%. Since cytology was not done on group II i.e. non-malignant ascites, specificity of cytology could not be calculated.

In conclusion, it is reiterated that biochemical markers in ascitic fluid are not elusive myths; they definitely have a role

Table 3 Comparative analysis of diagnostic value of cytology and biochemical analytes.

Assays	Definition of abnormality	Sensitivity	Specificity
Cytology	Malignant cells	40 (22.1–60.2)	–
AF LDH	Values above 422 U/L	74 (54.1–87.7)	60 (40.61–77.3)
AF cholesterol	Values above 67 mg/dL	70 (50.6–85.2)	60 (40.6–77.3)
AF ferritin	Values above 95 ng/mL	100	63 (43.8–80.1)
AF LDH, cholesterol, ferritin	Raised values beyond the cut-offs	61 (42.3–78.3)	93 (76.1–98.9)

to play in the diagnosis of malignant ascites. Cholesterol and LDH can be easily estimated in a peripheral hospital where the services of a pathologist to perform cytology are not available. Ferritin which is estimated by ELISA is a very good screening tool for malignant ascites. It is recommended that ascitic fluid ferritin should be done in all cases because of its high sensitivity; samples having values >95 ng/mL should be processed further as these are likely to be positive.

Intellectual Contributions of Authors

Study concept: Lt Col Mithu Banerjee

Drafting and manuscript: Lt Col Mithu Banerjee, Brig MM Arora

Statistical analysis: Mr D Bassannar, Seema Patrikar

Study supervision: Lt Col Mithu Banerjee

CONFLICTS OF INTEREST

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Best referee award 2010

The best referee award for 2010 was awarded to:

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- Lt Col AS Kushwaha, Associate Professor (Community Medicine), AFMC, Pune – 40
- Lt Col KJ Singh, Reader (Surgery), AFMC, Pune – 40
- Col DK Sreevastava, Senior Advisor (Anaesthesia), AH (R&R), New Delhi
- Col TS Ahluwalia, Senior Advisor (Ophthalmology), Command Hospital (NC)