

USEFULNESS OF ASCITIC FLUID CHOLESTEROL AND PROTEIN IN THE DIFFERENTIAL DIAGNOSIS OF ASCITES IN NIGERIA: COMPARISM WITH CONVENTIONAL CYTOLOGY.

ABSTRACT:

BACKGROUND: The differentiation of ascites into malignant and non-malignant group is a common problem in clinical practice. A simple biochemical marker is needed to differentiate between the two.

AIM: To assess and evaluate the diagnostic accuracy of cholesterol and protein in differentiating ascites into malignant and non-malignant group in comparism with cytology.

METHOD: A total of 75 consecutive patients of Nigerian origin with ascites (37 malignant and 38 non-malignant) were studied for cholesterol and protein concentration in ascites. Also, cytology was done for all the 75 samples of ascites.

RESULTS: The mean ascitic fluid cholesterol and protein levels in malignant ascites were higher (mean values of for cholesterol and 34.41 ± 16.97 g/L for protein respectively) than in non-malignant ascites (mean values of for cholesterol and for protein). The p value for cholesterol was less than 0.05. Using a cut-off limit of 72.7mg/dL, the sensitivity, specificity and overall diagnostic accuracy for cholesterol was found to be 84.2%, 100% and 94.7%. Ascitic protein had sensitivity, specificity and overall diagnostic accuracy of 37.8%, 86.8% and 62.7% respectively. Cytology had sensitivity, specificity, positive predictive value,

25 negative predictive value and overall diagnostic accuracy of
26 56.8%,100%,100%,70.4% 78.6% respectively. Cholesterol was more sensitive than
27 protein in the differentiating malignant from non-malignant ascites.

28 **CONCLUSION** :It was concluded that measurement of cholesterol in ascitic fluid
29 can differentiate between malignant and non-malignant ascites, and the
30 sensitivity would be higher when combined with cytology.

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32 **Keywords**

33 Cholesterol, protein, cytology ,malignant, non-malignant, ascites.

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38 **INTRODUCTION**

39 Ascites is defined as the abnormal accumulation of fluid in the peritoneal
40 cavity¹. The etiology of ascites is multifactorial and the pathophysiology
41 varies depending on its etiology^{2,3}. The presence of ascites can be related
42 to a malignant or non-malignant disease entity, and differentiation
43 between these two conditions is of considerable clinical significance for
44 further diagnostic and therapeutic procedure.

Cytological investigation of ascitic fluid is specific but may produce a large percentage of false-negative results. Its sensitivity ranges between 40 and 70%^{6,7}. Detection of malignant cells on cytology of ascitic fluid aspirates has been the cornerstone for diagnosis of malignant ascites. However, cytology does not have a good sensitivity³. In addition, reactive mesothelial cells in the ascitic fluid are mimics of malignant cells thus based on morphology above, it is difficult to distinguish between the two⁸. Many biochemical markers have been attempted to make a distinction between malignant and non-malignant ascites. One of such is ascitic fluid cholesterol. Cholesterol is a component of the cell membrane. When fragile malignant cells breakdown in the ascitic fluid, cholesterol from the cell membrane is released into ascitic fluid.^{5,9}. The present study was performed to distinguish malignancy-related ascites from non-malignant ascites using biochemical parameters as ascitic fluid cholesterol and protein and comparing with conventional cytology.

PATIENTS AND METHODS

Abdominal paracentesis was done for all patients within 24hrs of admission into the hospital. This was done prior to any form of surgical or medical intervention.

Samples of various blood were obtained at the same time of collecting ascetic fluid. The ascitic fluid was collected by aseptic method. Using a 22-gauge needle, about 20mls of ascetic fluid was aspirated from the left

lower quadrant of the abdomen. The aspirated ascitic fluid was immediately sent for assay of cholesterol, protein and cytology. Also 5mls of venous blood was obtained from the patients at the same time. The blood collected was also assayed for cholesterol and protein.

The protein in serum and ascitic fluid was assayed by biuret colorimetric method. This was done by a commercially available protein that (Randox laboratories, UK). The principle behind this test is based on the fact that the presence of peptide which reacts with copper ions in alkaline solution to form a colored product whose absorbance is measured spectrophotometrically at a wavelength of 540nm. The intensity of the colored product is proportional to the number of peptide bonds that react; and by inference equal to the level of protein in the reacting system.

Patients and Methods

We studied a total of 75 consecutive patients who were admitted through the gastrointestinal clinic, surgical clinic and obstetric/gynecologic clinic of the Lagos University Teaching Hospital (LUTH). This was between August 2011-February 2012. This involved adult consecutive patients with clinically detectable ascites. The mean age of the study group was 46.58 ± 12.44 years with a range of 18 years to 65 years. and ethical approval was given by the Health Research and Ethics Committee of the Lagos University Teaching Hospital. Signed informed consents were obtained from the patients. The subjects were divided into two groups- groups 1 (activity of 37 patient-made up of those with malignancy related

ascites) and Group II (made up of 28 patients in made up of those with non-malignant ascites.

Group 1 was made up of 7 males and 30 females, while group II had 18 males and 20 females. Total males participating in the study was 25 (33.3%) and 50 female (66.7%). The etiological distribution of these 37 patients was primary liver cell carcinoma 10(27.1%), cancer of the cervix, 5(13.5%), ovarian cancer, eleven 29.7%. cancer of the bladder, 1 (2.7%), hepatocellular cancer, 2 (5.4%), endometrial cancer, 1 (2.7%).

Cholesterol concentration was determined enzymatically with a commercial test (cholesterol CHOD PAP) made by Biolabo, Maizy, France).

Cytology was done for all collected samples of ascitic fluid to distinguish the group with malignancy from those that were non-malignant. Cytology was done using papanicolaou and giemsa stain smears made from sediments of centrifuged ascitic fluid within 2 hours of aspiration of the ascitic fluid.

Cytology involved the usual steps of tissue processing as sample collected (in this case ascitic fluid), was rolled over the slide and the smear fixed immediately. This was then stained with papanicolaou stain and later viewed under the microscope. The sample collected may also be air-dried, fixed in methanol and stained by using the modifications of May-graunwald stain. Examined slides under the microscope, if positive for malignancy showed the presence of malignant cells of various sizes, abnormal nuclei/cytoplasmic ratio, large nucleoli, abnormal mitosis and sometimes



114 with presence of numerous spherical clusters .A non-malignant ascites did
 115 not have the above-mentioned features. Infective processes like
 116 tuberculosis presented with mononuclear cells, macrophages and absence
 117 of malignant cells. Cytology was done for all the samples to determine if
 118 they were positive for malignancy or not.



119 Cytology was then compared with an already diagnosed malignancy based
 120 on a combination of clinical history/details, signs and symptoms, biopsy for
 121 histology of the organ/tissue affected by the cancer, radiological(computed
 122 tomography/abdominal scan) or autopsy. Based on this, the patients were
 123 divided into two groups.

124 Statistical Analysis

125 Results are expressed as mean± standard deviation. Student's t-test was
 126 used for statistical analysis of the data and $p < 0.005$ was considered
 127 statistically significant. Receiver operator characteristics (ROC) curve were
 128 calculated by standard procedures¹⁰. The ideal cut-offs for all the analyses
 129 were established differ generating the ROC curve using SPSS.



130 RESULTS

131 The total number of patients included in the study was 75. A total of 25
 132 (33.3%) were males and 50 (61.7%) were females with a mean age of
 133 46.58 ± 12.44 years. Patients in group 1 were slightly older than those in

134 group II. The distribution of ascites among different tribes is given by
135 figure 1. The highest tribe was represented by the was Yoruba (75%).

136 The age distribution of subjects with malignant and non-malignant ascites
137 is given below in table II. Liver cirrhosis is the commonest cause of ascites
138 17(48%). The mean body mass index (BMI) was $24.56 \pm 3.49 \text{ kg/m}^2$. More
139 than half of the study population (57.3%) were either traders,
140 businessmen/women, formers, unskilled people by profession.

141 The mean ascitic fluid protein was $34.41 \pm 16.67 \text{ g/l}$ and $71.5 \pm 7.35 \text{ g/l}$ for
142 plasma total protein. The sensitivity of ascitic protein was 62.7% at a cut
143 off value of 41.5g/l. The levels of protein in group I (Malignant) was
144 $38.72 \pm 18.00 \text{ g/l}$ and $30.21 \pm 15.50 \text{ g/l}$ for the non-malignant group (group
145 II). There was no significant statistical difference between the values of
146 protein from the two groups.

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150 **Table 1**

Ascitic fluid analyte	Area under the curve	P-value
Protein	0.629	P = 0.561
Cholesterol		P<0.005

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Comparative analysis of diagnosis value of cytology and biochemical analysis. Diagnostic value of cytology, ascitic fluid cholesterol, ascitic protein and their ability to distinguish between malignancy-related ascites and non-malignant ascites are summarized below in table II

As illustrated by the receiver operating characteristics (ROC) curve, the differential diagnostic accuracy of ascitic fluid of cholesterol and protein are shown below

The ascitic fluid cholesterol, and protein at discriminate points of 64g/l, and 41.5g/l respectively; separated patients with malignancy from patients with non-malignant ascites with accuracy of 85% and 62.7% respectively.

Table II

Serum analyte	Area under the curve	P-value
Protein	0.457	P = 0.521
Cholesterol	0.963	P<0.005

Table III

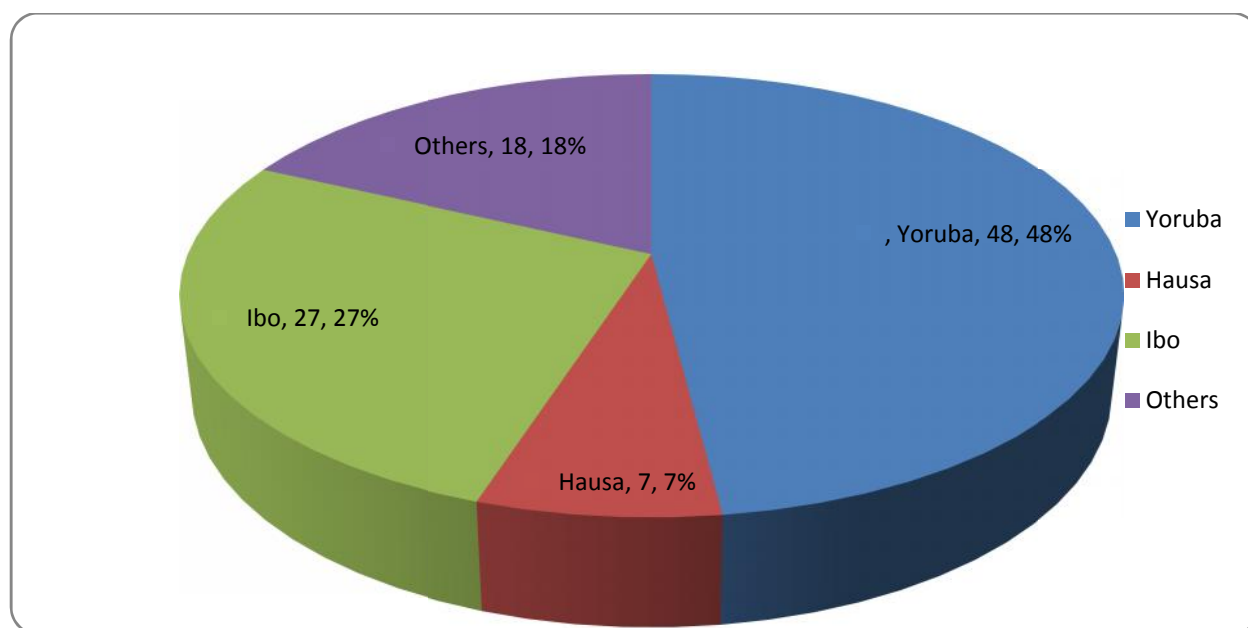
Variable	Sensitivity	Specificity	Accuracy	Positive Predictive	Negative Predictive	Cut-off

				Value	Value	value
Protein	37.8	86.8	62.7	73.7	58.9	41.5
Cholesterol	60	70	85	60	70	72.7
Cytology	56.8	100	78.6	100	70.4	

Table IV

Parameter	Malignant Ascites(x±SD)	Non-Malignant Ascites (x±SD)
Protein (g/l)	38.72±18.00	30.21±15.00
Cholesterol (mg/dl)		

FIG. 1



DISCUSSION

Ascites is a common clinical problem seen in many hospitals in Nigeria. It is caused by various diseases, the most common of which are chronic liver disease and malignancy¹¹. Many attempts have been made to differentiate ascites of malignant origin from non-malignant ascites by means of laboratory tests¹². This discrimination of malignant ascites is of paramount importance because the therapy and management of the two groups is radically different¹³. Hitherto, the use of cytology to differentiate between malignant ascites and non-malignant ascites has met with limitations. This has necessitated the need for other biochemical parameters

Calstaldo et al have cited a sensitivity of 40-60% for cytology in their study¹⁴. In this study, the sensitivity of cytology was 56.8%. A total of 75

consecutive patients were studied with ascites. 37 had malignant ascites. Levels of protein were higher in malignant ascites than in non-malignant ascites, however, the sensitivity was low (37.8%). This agrees with earlier works done by Aksoyet al¹³. This finding supports the view of various researchers that protein levels are not definite criteria for differentiating malignant from non-malignant ascites.

Rolvstad et al reported a high lipid concentration in the ascites of malignant origin¹⁵. This was later emphasized by other subsequent researchers^{16,17,18}. The cholesterol levels obtained in the malignant ascites was high. This is also consistent with other researchers. In this index study, using a cut off of 64mg/dl, the sensitivity of cholesterol was 85% while the specificity of cholesterol was 70%. Prieto et al reported an mean asciticfluid cholesterol of 109.06±29.85 mg/dl in the group with malignant ascites as against 23.75±11.22 mg/dl in the non-malignant group¹⁷.Laudanno et al also reported an efficacy of 98% in differentiating ascites caused by chronic liver diseases or by malignancy, using cholesterol as a biomarker¹⁹.

Increased levels of cholesterol in ascitic fluid due to malignancy are thought to be of various etiologies. Based on previous studies, it was thought that the high cholesterol may be originating from malignant cell membrane²⁰. Other authors are of the view that it could be due to tumor involvement of the serosal cavity²¹. It may enter the cavity from the interstitial space because of obstructed lymph vessel or may be carcinomatosis serous membrane¹³.

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