

## **Usefulness of Ascitic Fluid Cholesterol and Protein in the Differential Diagnosis of Ascites in Nigeria: Comparison with Conventional Cytology**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author EELE designed the study, managed the literature searches, performed the statistical analysis with Prof E.E. Ekanem, wrote the protocol and wrote the first draft of the manuscript. Author GAE managed the analyses and supervision of the study. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** This study was done to assess and evaluate the diagnostic accuracy of cholesterol and protein in differentiating ascites into malignant and non-malignant group in comparison with cytology.

**Study Design:** A cross sectional study to determine the correlation between ascitic fluid cholesterol and protein and malignant and non-malignant ascites.

**Place and Duration of Study:** This study was carried out at the clinics of gastroenterology,

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surgery, and obstetrics/gynecology at the Lagos University Teaching Hospital (LUTH), between August 2011 and July 2013.

**Methodology:** A total of 75 consecutive patients of Nigerian origin with ascites (37 malignant and 38 non-malignant) were studied for total cholesterol and total protein concentration in ascites. Also, cytology was done for all the 75 samples of ascitic fluid. Statistical analyses were carried out using SPSS software (version 15.0), and the level of significance set at  $p < 0.05$  and  $p < 0.001$ .

**Results:** The ascitic fluid cholesterol and protein levels in malignant ascites were higher (values of  $103.10 \pm 30.00$  mg/dL for cholesterol and  $38.72 \pm 18.00$  g/L for protein respectively) than in non-malignant ascites (values of  $33.20 \pm 22.00$  mg/dL for cholesterol and  $30.21 \pm 15.00$  g/L for protein). The  $p$  value for cholesterol was less than 0.001. Cytology had sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of 56.8%, 100%, 100%, 70.4%, and 78.6% respectively. Using a cut-off limit of 72.7 mg/dL, cholesterol had sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of 94.6%, 94.7%, 94.6%, 94.7% and 94.7% respectively. Ascitic fluid total protein had sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of 37.8%, 86.8%, 73.7%, 58.9%, and 62.7% respectively. Cholesterol was more sensitive than protein and cytology in the differentiating malignant from non-malignant ascites.

**Conclusion:** It was concluded that measurement of cholesterol in ascitic fluid can differentiate between malignant and non-malignant ascites, and can supplement cytology in the differential diagnosis of ascites.

*Keywords: Cholesterol; protein; cytology; malignant; non-malignant; ascites.*

## 1. INTRODUCTION

Ascites is defined as the abnormal accumulation of fluid in the peritoneal cavity [1]. The etiology of ascites is multifactorial and the pathophysiology varies depending on its etiology [2,3]. The presence of ascites can be related to a malignant or non-malignant disease entity; and differentiation between these two conditions is of considerable clinical significance for further diagnostic and therapeutic procedure [4,5]. The differentiation of ascites into malignant and non-malignant group is a common problem in clinical practice confronting physicians [6]. A simple biochemical marker is needed to differentiate between the two. Cytology is one common investigation used to differentiate malignant from non-malignant ascites.

Cytological investigation of ascitic fluid is specific but may produce a large percentage of false-negative results. Its sensitivity ranges between 40 and 70% [7,8]. 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 sensitivity [3,7,8]. 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 two [9]. 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 [5,10]. The present study was carried out to distinguish malignancy-related ascites from non-malignant ascites using biochemical parameters such as ascitic fluid cholesterol and protein and comparing with conventional cytology.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This was a cross sectional study involving 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 and July 2013. This involved adult consecutive patients with clinically detectable ascites. The mean age of the study group was  $46.58 \pm 12.44$  years with a range of 18 years to 65 years and ethical approval (REF. NO:ADM/DCST/HREC/VOL.XVI/101) was given by the Health Research and Ethics Committee of the Lagos University Teaching Hospital. This study has been examined and approved by the appropriate ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki. 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 38 patients with non-malignant ascites). Inclusion criteria was all patients with obvious ascites from any cause while patients with recent abdominal paracentesis, patients on cytotoxic drugs, non-compliant patients and immunosuppressed patients were excluded from the study.

## 2.2 Sample Collection

Abdominal paracentesis was done for all patients within 24 hours of admission into the hospital. This was done prior to any form of surgical or medical intervention. Samples of 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 20 mls 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, 5 mls of venous blood was obtained from the patients at the same time. The blood collected was also assayed for cholesterol and protein.

## 2.3 Laboratory Analysis

The protein in serum and ascitic fluid was assayed by biuret colorimetric method. This was done by a commercially available protein kit (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 540 nm. 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 [11].

Ascitic fluid cholesterol concentration was determined enzymatically by routine method with a commercially available cholesterol reagent (cholesterol CHOD PAP) made by Biolabo, Maizy, France). The principle is that cholesterol esters are hydrolysed by cholesterol ester hydroxylase to free cholesterol and fatty acids. The cholesterol is eventually converted to cholestenone and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase converts the chromogen (4aminophenazone) to a red colour, whose wavelength is measured at 510 nm and is proportional to the concentration of the cholesterol [12,13].

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 stained smears made from sediments of centrifuged ascitic fluid (at 12,000 rpm) 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. 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 with presence of numerous spherical clusters. A non-malignant ascites did not have the above-mentioned features. Infective processes like tuberculosis presented with mononuclear cells, macrophages and absence of malignant cells. Cytology was done for all the samples to determine if they were positive for malignancy or not. Cytology was then compared with an already diagnosed malignancy based on a combination of clinical history/details, signs and symptoms, biopsy for histology of the organ/tissue affected by the cancer, radiological (computed tomography/abdominal scan) or autopsy. Based on this, the patients were divided into two groups.

## 2.4 Statistical Analysis

Results are expressed as mean± standard deviation. Student's t-test was used for statistical analysis of the data and  $p < 0.05$  and  $p < 0.001$  were considered statistically significant. Receiver operator characteristics (ROC) curves were calculated by standard procedures [14]. This was created by plotting the fraction of true positive rate (sensitivity) against the false positive rate (1-specificity). The area under the curve is a relative measure of the diagnostic test performance. The ideal cut-offs for all the analyses were established by generating the ROC curve using SPSS.

## 3. RESULTS

The total number of patients included in the study was 75. A total of 25 (33.3%) were males and 50 (61.7%) were females with a mean age of  $46.58 \pm 12.44$  years. Patients in group 1 were slightly older than those in group II. The distribution of ascites among different tribes is given by Fig. 1.

The highest tribe was represented by the Yorubas (75%).

This probably may be due to fact that this study was carried out in a Western Nigerian state notably dominated by the Yoruba tribe.

The age distribution of subjects with malignant and non-malignant ascites is given below in Table 2. Liver cirrhosis was the commonest cause of ascites, 17(48%) among these patients. The mean body mass index (BMI) was  $24.56 \pm 3.49 \text{ kg/m}^2$ . More than half of the study population (57.3%) was traders, businessmen/women, farmers, and unskilled people by profession. 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 females (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%).

The mean ascitic fluid protein was  $34.51 \pm 16.50 \text{ g/l}$  for plasma total protein and  $68.15 \pm 26.00 \text{ mg/dL}$  for mean ascitic cholesterol. The sensitivity of ascitic protein was 37.8 % at a cut off value of 41.5 g/l. The levels of protein in

group I (Malignant) was  $38.72 \pm 18.00 \text{ g/l}$  and  $30.21 \pm 15.00 \text{ g/l}$  for the non-malignant group (group II). There was no significant statistical difference between the values of protein from the two groups. The sensitivity of ascitic cholesterol was 94.6% at a cut off value of 72.7 mg/dL. Level of ascitic cholesterol in the malignant group was  $103.10 \pm 30.00 \text{ mg/dL}$  and  $33.20 \pm 22.00 \text{ mg/dL}$  for the non-malignant group. There was a statistical difference between the values of ascitic cholesterol for the two groups ( $p < 0.001$ ). The ascitic fluid cholesterol and protein at discriminate points of 72.7 mg/dL, and 41.5 g/l respectively; separated patients with malignancy from patients with non-malignant ascites with accuracy of 94.7% and 62.7% respectively. Levels of ascitic fluid cholesterol and protein were higher than plasma cholesterol and protein respectively. The areas under the ROC curves were highly statistically significant (see Tables 1 and 4). There was no correlation between body mass index and any assayed parameter; also correlation did not exist between patients' profession and the assayed parameters.

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 3.

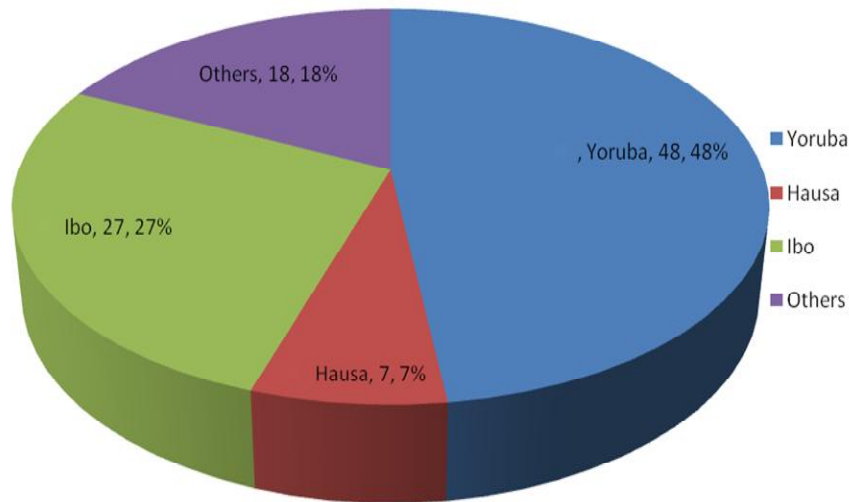
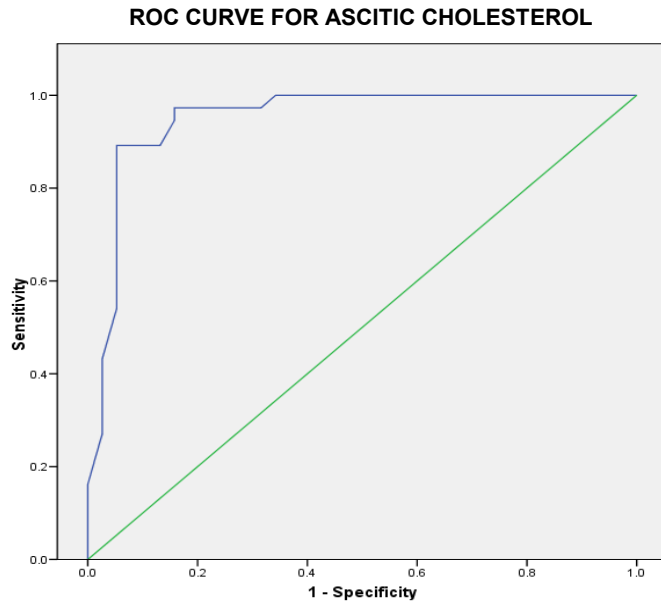


Fig. 1. PIE chart showing distribution of patients by tribes

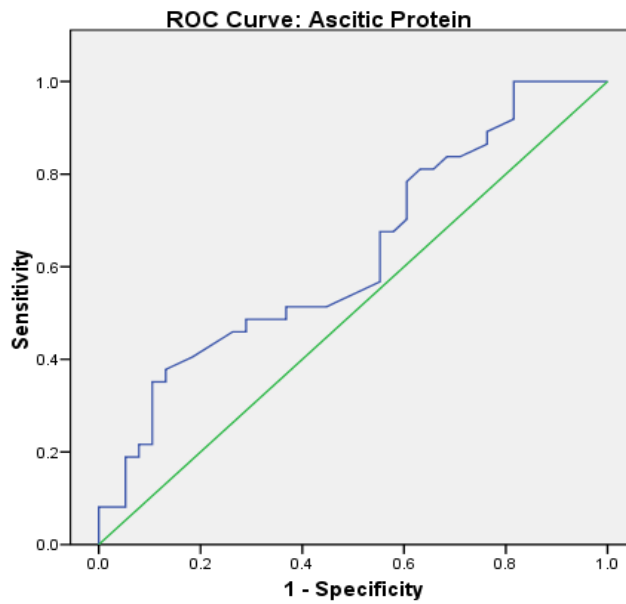
Table 1. Receiver operating characteristic (roc) curve analysis of ascitic fluid variables

Ascitic fluid analyte	Area under the curve	P-value
Protein	0.629	$P = 0.061$
Cholesterol	0.989	$P < 0.001$

P value is significant at  $p < 0.05$  and  $0.001$ . NMA=37; MA=38. \*significant



**Fig. 2. ROC curve for ascitic cholesterol**



**Fig. 3. ROC curve for ascitic protein**

**Table 2. Receiver operating characteristic (ROC) curve analysis of plasma variables**

Serum analyte	Area under the curve	P-value
Protein	0.457	$P = 0.521$
Cholesterol	0.963	$P < 0.001$

*P* value is significant at  $p < 0.05$ ; 0.001. NMA=37; MA=38. \*significant

As illustrated by the receiver operating characteristics (ROC) curve, the differential diagnostic accuracy of ascitic fluid of cholesterol and protein are shown below by the analysis and diagrams in Table 1 and Figs. 2 and 3. Respectively.

**4. 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 malignancy [15]. Many attempts have been made to differentiate ascites of malignant origin from non-malignant ascites by means of laboratory tests [16]. This study focuses on differentiating malignant and non-

malignant by laboratory indices. This discrimination of malignant ascites is of paramount importance because the therapy and management of the two groups is radically different [17]. 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. [18] have cited a sensitivity of 40-60% for cytology in their study. In this study, the sensitivity of cytology was 56.8%. Slides of malignant and benign ascites are shown in figures 4 and 5 respectively. In this index study, a total of 75 consecutive patients were studied with ascites.

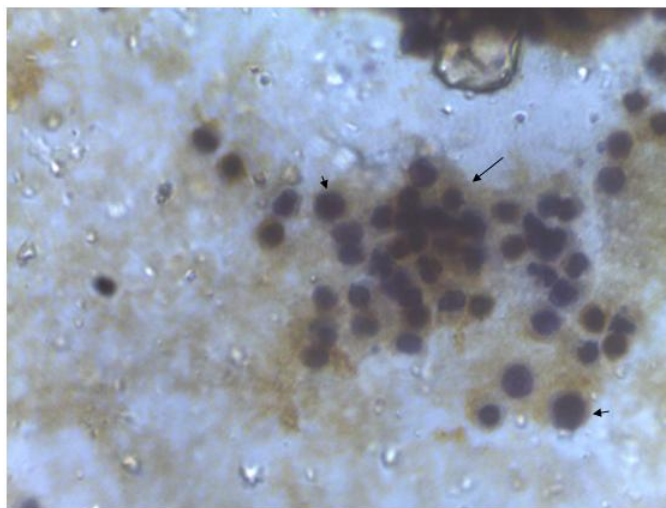
**Table 3. Sensitivity, specificity, accuracy and positive and negative values of variables in separating 37 patients with malignant-related ascites from 38 patients with non-malignant ascites (Total 75 patients)**

Variable	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Cut-off value
Protein	37.8	86.8	62.7	73.7	58.9	41.5 g/L
Cholesterol	94.6	94.7	94.7	94.6	94.7	72.7 mg/dL
Cytology	56.8	100	78.6	100	70.4	

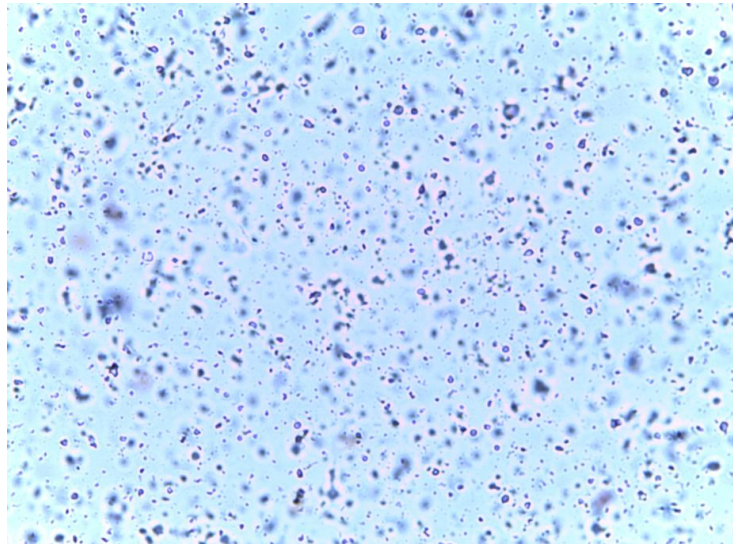
**Table 4. Results of analysis of malignant and non-malignant ascitic fluid**

Parameter	Malignant Ascites (x±SD)	Non-Malignant Ascites (x±SD)
Protein (g/l)	38.72±18.00	30.21±15.00
Cholesterol (mg/dL)	109.06±29.85	23.75±11.22*

*P value is significant at p<0.05; 0.001. NMA=37; MA=38. \*significant*



**Fig. 4. Slide showing malignant cytology—evidenced by discohesive clusters of malignant cells(long arrow),increased nucleocytoplasmic ratio, irregular nuclei,hyperchromasia and coarse chromatin pattern(arrow head)**



**Fig. 5. Slide showing features of benign ascitic cytology**

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 finding supports the view of various researchers that protein levels are not definite criteria for differentiating malignant from non-malignant ascites [15]. The aetiology of elevated cholesterol in malignant ascites is due to increased vascular permeability, increased cholesterol synthesis and release from malignant cells implanted on the peritoneum [10,19-21]. In this index study, ascitic fluid cholesterol concentration was significantly elevated in malignant ascites when compared to non-malignant ascites. The cholesterol levels obtained in the malignant ascites was high. This is also consistent with other researchers [20-23,25]. In this index study, using a cut off of 72.7mg/dl, the sensitivity of cholesterol was 94.6% while the specificity of cholesterol was 94.7% and the diagnostic accuracy was 94.7%. This is supported by the study done by Sood et al. [21]. Rana et al. [20] at a cut-off value of 70 mg/dl had a diagnostic accuracy of 94%. Gupta et al. [21] used a cut-off value of 55 mg/dL and had a diagnostic accuracy of 94%. Rolvestad et al. [23] reported a high lipid concentration in the ascites of malignant origin. This was later emphasized by other subsequent researchers [22,24,25]. Prieto et al. [25] reported mean ascitic fluid cholesterol of  $109.06 \pm 29.85$  mg/dl in the group with malignant ascites as against  $23.75 \pm 11.22$  mg/dl in the non-malignant group. Laudanno et al. [26] also reported an efficacy of 98% in differentiating ascites caused

by chronic liver diseases or by malignancy, using cholesterol as a biomarker. Bansal et al. [27] also concluded that ascitic fluid cholesterol and lactate dehydrogenase were best for diagnosing malignant ascites. 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 membrane [28,29]. Other authors are of the view that it could be due to tumor involvement of the serosal cavity [17]. It may enter the cavity from the interstitial space because of obstructed lymph vessel or may be carcinomatous serous membrane [17,30].

## 5. CONCLUSION

This study focused on the usefulness of ascitic fluid cholesterol in differentiating malignant and non-malignant ascites. Cholesterol assay is cheap, easy and readily done. Its application in this differentiation can be very useful in resource-poor environment. Ascitic fluid cholesterol may be relevant in the differential diagnosis of ascites especially if supplemented with cytology. Further studies in larger number of subjects may validate these findings.

## STRENGTH OF THE STUDY

This study has been able to demonstrate a strong relationship between ascitic fluid cholesterol and malignancy.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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