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Differentiating malignancy-related ascites from cirrhotic ascites: Diagnostic significance of serum and ascitic fluid cholesterol, albumin, protein, fibronectin serum ascites albumin gradient (SAAG), serum ascites cholesterol gradient (SACG)*

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ABSTRACT

Background: Over the years, differential diagnosis of ascitic fluid has been a dilemma for practicing physicians. Many analytes have been assessed to enhance diagnosis in this regard. In developing countries, the emphasis is laid on using less expensive biochemical parameters and methods to differentiate ascitic fluid. The aim of the present study was to assess the value of ascitic fluid cholesterol, albumin, protein, and their gradients [serum ascites albumin gradient (SAAG) and serum ascites cholesterol gradient (SACG)] in differentiating malignant and cirrhotic ascites.

Materials and Methods: This cross-sectional prospective study was carried out over a 2-year period at the medical, surgical, emergency, and gynecology units of the Lagos University Teaching Hospital. A total of 61 adult patients with ascites (35 males, 26 females) were recruited for the study. Serum and ascitic fluid were assessed for levels of cholesterol, protein and albumin and their gradients.

Results: Of the total 61 adult patients recruited (35 males, 26 females), the mean age of the study population was 46.84 ± 12.10, mean body mass index was 24.43 ± 3.18. Serum and ascitic values of cholesterol, protein, and albumin were 125.1 ± 79.30, 72.29 ± 6.65, and 39.51 ± 7.98 mg/dl, with corresponding p values of $p = 0.0475$, $p = 0$, and $p = 0.072$. The accuracy of serum cholesterol, protein, and albumin was 81.99%, 37%, and 60.6% when compared to ascitic cholesterol, protein, and albumin (58.9%, 70.5%, and 67.2%). Serum protein had a sensitivity of 94.3% while ascitic cholesterol had a sensitivity of 51.4%. Mean SACG and SAAG were 89.84 ± 79.89 and 11.48 ± 8.7, respectively.

Conclusion: Differentiation of cirrhotic and malignancy-related ascites seem to have very little diagnostic values from analytes of cholesterol, albumin, and protein.

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KEYWORDS

Cholesterol; ascitic; cirrhosis; malignant; albumin; protein; SAAG; SACG; gradient

Introduction

Abnormal effusion and accumulation of fluid into the peritoneal cavity is termed ascites, and it is usually a complication of different diseases [1]. Being a common medical problem, it is usually seen by many physicians globally [1]. The aetio-pathologies involve virtually almost all the systems of the body. Liver cirrhosis accounts for more than 80% of all ascitic cases seen globally. Other causes of this clinical sign include malignant peritonei, congestive heart failure, nephrotic syndrome, and chronic kidney diseases among others [2,3].

A common problem, however, is the differential diagnosis of ascites. This is highly important for therapeutic reasons. Different analytes in serum and ascitic fluid have been studied globally to enhance the differential diagnosis of ascites. Some of these analytes include amylase, cholesterol, protein, albumin, fibronectin, adenosine deaminase, and lactic dehydrogenase among others. However, none has yet proved 100% effective in diagnosing the etiology of ascitic fluid [3–7]. Serum ascites albumin gradient (SAAG) has been proposed to be accurate in differentiating exudates from transudates [6,8–12].

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Many studies have been carried out to assess the place of cholesterol in the differential diagnosis of ascitic fluid [12–18]. Most of the studies carried out on this subject matter focused attention on Asians and Caucasian. Few studies have been done on African subjects. Since the majority of the ascite cases are of cirrhotic origin, the aim of this study was to propose analytes that could differentiate cirrhosis-related ascitic fluid from the malignant-based ascitic fluid. The paucity of published materials in our environment in this regard prompted my interest in doing this research in an African setting.

This study focuses on assessing the differentiation of liver cirrhotic-based ascites from malignant ascites using serum ascites cholesterol gradient (SACG), SAAG, albumin, protein, and cholesterol.

Materials and Methods

Study design

This prospective, cross-sectional study lasted for a period of 24 months. The setting for this study was at the Lagos University Teaching Hospital of patients attending gastroenterology, surgical, and radiotherapy clinics.

Inclusion criteria

- Patients aged between 18 and 65 years
- Patients willing to participate in this study
- Patients who were not immuno-compromised
- Patients not on chemotherapy and radiotherapy
- All patients admitted within 24 hours of the hospital
- All patients with ascitic fluid based on clinical examination and abnormal ultrasound.

Exclusion criteria

- Patients unwillingness to participate in the study
- Ascitic fluid patients on therapy or medications.

A total of 61 willing subjects were recruited (35 males, 26 females). The ethical committee granted approval for this study [administration (ADM)/ director of clinical training (DCT)/ human resources and ethical committee (HREC) XL.XIV/101]. Informed consent was sought from all participants. Under aseptic methods, after careful explanation, abdominal paracentesis was done to obtain ascitic fluid following standard guidelines procedures described by American Association for Liver Diseases [19]. From each patient, 20 ml of ascitic fluid was collected within the first 24 hours of admission, prior to any medical intervention. The sample obtained was centrifuged at 10,000 rpm for a period of 5 minutes at room temperature. Concurrently, each patient had 5 ml of venous blood collected while collecting abdominal ascitic fluid. This was done after an overnight fast of about 8–12 hours. This blood sample was centrifuged to get serum and then assayed for serum cholesterol, albumin, total protein, and SAAG, and SACG. The collected sample was stored at –20 °C till assayed. Both ascitic and serum samples were assayed for albumin, cholesterol, and protein. Both SAAG and SACG were calculated as:

SACG = Serum cholesterol – Ascitic fluid cholesterol

SAAG = Serum albumin – Ascitic fluid albumin

Serum and ascitic fluid albumin were estimated by Bromocresol green method [20]. Protein in serum and ascitic fluid was assayed by the Biuret method [21]. Cholesterol was by an enzymatic method (Cholesterol oxidase) [22]. Malignancy-related and cirrhotic-based ascites were diagnosed by a combination of history, clinical examination, cytology, radiological investigation, and ultimately by histological examination of the representative sample following a liver biopsy.

Statistical analysis

Data collected were analyzed using SPSS version 20. Receiver operating characteristic was plotted to determine the specificity and sensitivity. Unpaired Student *t*-test was used to compare mean values of the two groups (malignant and liver-cirrhotic). All data were expressed as mean ± standard deviation (SD). A tailed probability was taken with a value of <0.05 being taken as significant.

Results

The study included 61 adult patients with ascites, based on different etiologies. Among the 61 patients, 26 (12 males, 14 females) of them had ascites secondary to cirrhosis, and 35 of them (seven males, 28 females) had malignancy-related ascites. A breakdown of the malignancy-based ascites showed that: 10 had primary liver cell carcinoma, 10 had ovarian cancer, 10 had breast cancer, three had renal cell carcinoma, one had cervical cancer, and one had cancer of the bladder. A total of 19 males (31%) and 42 females (69%) took part in this study. The mean ± SD age of the study population was 46.84

± 12.10, mean body mass index ± SD was 24.43 ± 3.18. The mean plasma values of cholesterol, total protein, and albumin were 125.1 ± 79.30 mg/dl, 72.29 ± 6.65 mg/dl, and 39.5 ± 7.98 mg/dl, respectively. The corresponding values of ascitic fluid cholesterol, total protein, and albumin for malignant ascites were noted and are represented in Table 1.

Also, the mean ascitic fluid fibronectin level for malignant ascites patient was 97.54 ± 17.73 µmol/l and 47.76 ± 15.32 µmol/l for cirrhotic patients. The mean SAAG was 6.74 ± 0.1 and 13.5 ± 1.0 for both malignant and cirrhotic ascites patients, respectively, while SACG was 42.11 ± 1.31 mg/dl and 93.7 ± 2.73 mg/dl for both malignant and cirrhotic ascites, respectively (see Table 1).

Using various cut-off values, the diagnostic accuracy of SAAG, SACG, ascitic fluid cholesterol, and fibronectin in differentiating malignancy-related ascitic fluid from cirrhotic ascites were given as 73.3%, 93%, 94.7%, and 94.7%, respectively. The values of sensitivity, specificity, negative predictive value, positive predictive value, and their various cut-off values are all represented in Table 2. This was obtained after a Receiver–Operator Characteristic curve was plotted.

The accuracy of protein (total) and albumin is 62.5% and 50.7% at cut off values of 42 and 49 mg/dl, respectively, with sensitivities of and for both ascitic total protein and albumin, respectively.

Discussion

Ascites is a common complication of most patients with liver cirrhosis [23]. Its prevalence could be as high as 75% in patients with liver cirrhosis [24,25], while malignancy-related ascites account for about 10% of ascites [25]. With the increasing burden of liver diseases globally, it is expected that adequate diagnosis is very vital to curb the complications of liver diseases. Viral hepatitis was the commonest cause of cirrhosis in these patients studied with cirrhosis, while malignancy-related ascites was mainly due to hepatocellular carcinoma and metastases from other intra-abdominal tumors to the peritoneum.

In this index study, attempts are made to locate discrete and sensitive common biomarkers that can distinguish malignant from cirrhotic ascites. This is highly vital because the therapeutic approach is different for these two forms of ascites [26]. Many malignant-based ascites have high cholesterol, and this stems from the fact that elevated cholesterol levels found in ascitic fluid is due to increased permeability of the blood vessels, increased production of cholesterol and the release of cholesterol and other lipids from malignant cells which exude into the peritoneum [27–29]. In this study, the levels of cholesterol obtained in the malignant ascitic fluid were elevated when compared to values obtained in cirrhotic patients.

Using a cut-off value of 72 mg/dl, the sensitivity and accuracy of cholesterol in differentiating malignant from cirrhotic ascites were 94.6% and 94.7%, respectively. This agrees with the finding of other researchers [28,29,30,31]. Rana et al., Gupta et al., and Laudano et al. obtained a diagnostic accuracy of 94%, 94%, and 98% in their studies, respectively. The total protein both malignant and cirrhotic ascitic fluid was not statistically significant

Table 1. The analytes in ascitic fluid in both malignant and cirrhotic ascites and their *p*-values.

Parameter	Malignant ascites (mean ± SD)	Cirrhosis (mean ± SD)	*p*-values
Ascitic fluid cholesterol (mg/dl)	80.07 ± 28.81	24.69 ± 9.28	$p < 0.001$
Ascitic fluid protein (mg/dl)	38.72 ± 18.00	30.22 ± 15.20	Not significant
Ascitic fluid albumin (mg/dl)	29.11 ± 7.22	32.33 ± 10.07	Not significant
Ascitic fibronectin (µg/ml)	97.54 ± 17.73	47.77 ± 15.32	$p < 0.05$
SAAG	6.74 ± 1.01	13.5 ± 2.04	$p < 0.05$
SACG mg%	54.11 ± 16.30	90.17 ± 13.45	$p < 0.005$

Table 2. The sensitivity, negative predictive value (NPV), positive predictive value (PPV), specificity and accuracy of the ascitic fluid analytes at various cut-off values.

Parameter	SAAG	SACG	Ascitic cholesterol	Ascitic fibronectin	Total protein	Albumin
Sensitivity (%)	59.5	86	94.6	94.6	37.8	0.0
NPV (%)	68.8	68.8	94.6	94.6	85.7	100
PPV (%)	81.5	81.5	94.6	94.6	73.7	0
Specificity (%)	86.5	86.5%	94.7	94.7	86.8	100
Accuracy (%)	73.3	90	94.7	94.7	62.5	50.7
Cut-off value	<11.5 g/dl	<50 mg%	72.7 mg/dl	73 µg/ml	42 mg/dl	49 mg/dl

(39.21 ± 13.1 *vs*. 30.17 ± 11.0, $p > 0.005$). Various studies have been done and many conclude that total protein may have no relevance in the discrimination of malignant from the cirrhotic ascitic fluid. The sensitivity and accuracy of total protein were 63% and 39%, respectively [28,32,33].

Fibronectin was able to differentiate the malignant from cirrhotic ascites (98.5 ± 10 µg/ml *vs*. 43.3 ± 5 µg/ml, $p < 0.05$). This also supports the findings of other people who had elevated fibronectin levels in the malignancy-based ascitic fluid [34–36]. Fibronectin in high amounts in malignant ascites linked to being from neoplastic cells. Fibronectin is usually elevated in certain malignancies like hepatocellular carcinoma, ovarian tumors, metastatic cancer, and peritoneal carcinomatosis [37,38]. In this study, the diagnostic accuracy of fibronectin in differentiating cirrhotic from malignancy-based ascites was 94.7%. This confirms high accuracy of the studies of Ghilain et al., Lee et al., and Scholmerich et al. [34–36,39–42].

SACG at a cut-off value of <50 mg% showed a sensitivity and accuracy of 86% and 93%, respectively. Again, this is in tandem with the findings of Sastry et al. [43] and Sapra et al. [43] whose sensitivity and accuracy in their studies were 94% and 90%, respectively, but was contrary to the study of Dharwardkar et al. [16] who had an accuracy of 68% and sensitivity of 60.4% at a cut-off value of less than 95 mg% for SACG .

SAAG has been known to be widely applicable in the differential diagnosis of ascites. The SAAG value was 13.5 ± 0.1 in the cirrhotic ascites *vs.* 6.75 ± 0.1 in the malignant ascites ($p < 0.005$) with an accuracy of 73.3%. This is similar to the findings of Lee et al. [41], Sharatchandra[44], Runyon et al. [45], Laudano et al. [33], and Nadeem et al. [33,46].

Conclusion

From the above study, it is obvious that ascitic values of cirrhotic ascitic fluid cholesterol, SAAG, SACG, and ascitic fluid fibronectin are well able to differentiate ascitic conditions of cirrhosis and neoplastic. The advantage here is that in resource-poor environments, these analytes may be used in the differential diagnosis of ascites while awaiting histology to give a definitive diagnosis.

This enables the physician to commence early therapy before getting all the final results for further management. This is in view of their high diagnostic accuracy, and their low cost of assay.

Conflict of interest

None declared.

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