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Ascitic Fluid Fibronectin: A Marker to Differentiate Between Malignant and Non-malignant Ascites

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Authors' contributions

This work was carried out in collaboration between all authors. Author EELE designed the study, wrote the protocol, and wrote the first draft of the manuscript and did the data analysis with Prof Ekanem. Author ECA corrected the various drafts of manuscripts and author DMB supervised the experimental processes and the overall work. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background and Aims: So far, the differentiation between malignant and non-malignant ascites by laboratory parameters has not been fully achieved yet. Fibronectin is a glycoprotein which plays an important role in cell adhesion, growth, migration, and differentiation. The aim of the study was to assess the accuracy of fibronectin for the diagnosis of malignant ascites and to compare it with conventional use of cytology.
Study Design: A cross sectional study to determine the correlation between ascitic fluid fibronectin and malignant and non-malignant ascites.
Place and Duration of Study: This study was carried out at the clinics of gastroenterology, surgery, and obstetrics/gynecology at the Lagos University Teaching Hospital (LUTH), between August 2011 and July 2013.
Methods: Ascitic fluid and serum samples from 75 patients were taken. 37 of them (7 males and 30 females) had malignancy-related ascites (Group 1), while the other 38 (18 males and

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20 females) had non-malignant ascites (Group 2) respectively. These were analysed for fibronectin, lactate dehydrogenase (LDH), total protein, and albumin. Cytology was also done for all ascitic fluid samples.

Results: Mean values of ascitic fluid fibronectin and LDH were higher in malignancy-related ascites (97.5 µg/ml, and 900.60 IU/L) respectively than in non-malignant ascites (47.7 µg/ml, and 199.31 IU/L) respectively (*P* less than 0.001). Ascitic fluid fibronectin with a cut-off value of 73 µg/ml gave the best diagnostic accuracy with a sensitivity and specificity of 94.7% and 94.6% respectively, while ascitic fluid LDH with a cut-off value of 310 IU/L gave diagnostic accuracy with a sensitivity and specificity of 97.3% and 84.2% respectively. The mean total protein level in the malignant group was 38.72±18.00 g/L and 30.21±15.00 g/L for the non-malignant group. The mean albumin levels were 28.08±10.32 g/L and 31.23±10.01 g/L for the malignant and non-malignant groups respectively. For both total protein and albumin, the *P* value was statistically insignificant. In this study, cytology yielded a sensitivity of 56.8% and a specificity of 100%.

Conclusion: The results of this study suggest that fibronectin concentration in ascitic fluid may be useful in differentiating malignant from non-malignant ascites and could supplement cytology in the differential diagnosis of ascites. Further studies are needed to confirm these results.

Keywords: Fibronectin; cytology; lactate dehydrogenase; malignant and non-malignant; ascites.

1. INTRODUCTION

Ascites is an important clinical situation that can be described as the pathological accumulation of fluid in the peritoneal cavity [1,2]. It is a common clinical sign with a wide range of causes [3]. Though many theories try to explain the pathogenesis of ascites, the exact mechanism regarding the formation of ascites remains controversial 1. The most common reason for the appearance of ascites is cirrhosis [3], although tuberculosis peritonitis, congestive heart failure, pancreatic, and renal induced ascites also occur [4].

Differentiation between malignant and non-malignant ascites is a common clinical problem because no single routine biochemical laboratory test can completely distinguish between them [5] and, although cytological examination of ascitic fluid is highly specific, its diagnostic sensitivity is only about 40-60% [6,7].

There are no distinctive features and no single diagnostic test is accurate in differentiating malignant and non-malignant ascites [8] and a large percentage of false-negative results may be produced by cytological investigation [7,9].

Based on these, other parameters of ascitic fluid need to be investigated. These other parameters would therefore be helpful to speed up the diagnostic process as inconclusive or false negative results from analysis of ascites could lead a clinician to consider the need for a more invasive diagnostic application such as laparascopy [2,10].

Diagnostic performance has been improved by combining cytology with ascitic fluid analysis in some studies [11], but complete discrimination between malignancy-related and non-malignant ascites has never been achieved [5,8].

Recent investigations have drawn attention to the surface properties of cancer cells, suggesting new possible markers of malignant effusions [12]. Cholesterol and fibronectin have been found to be elevated in malignant ascites [3,8,13].

However, in comparing fibronectin and cholesterol in terms of sensitivity, fibronectin has been found to be 100% sensitive in differentiating between malignant and non-malignant ascites [3,8,13-14].

In addition, ascitic fluid fibronectin results can be obtained in less than three hours after paracentesis, thereby enhancing patients' short stay in hospital.

Furthermore, there is no published material on the differential diagnosis of ascitic fluid using fibronectin in the scientific literature in our environment to our knowledge.

Though, the usefulness of fibronectin among patients with sickle cell disease [15], malnutrition [16] and pregnancy [17] have been studied in our environment, but no mention of it has been made concerning its role in differentiating ascites. Few articles concerning the usefulness of ascitic fluid fibronectin have been documented worldwide. Even then, many of them are on Caucasians. This study may probably be the first to explore the role of fibronectin in differentiating malignant

from non-malignant ascites among adult Nigerians.

In this aspect, the aim of the current study is to try to find a discriminate model to distinguish malignancy-related from non-malignant ascites using fibronectin. Its aim is to determine the correlation between ascitic fluid fibronectin and malignant and non-malignant ascites.

2. PATIENTS AND METHODS

This is a cross-sectional study involving unselected patients with clinically detectable ascites who were admitted to Lagos University Teaching Hospital (LUTH). LUTH is a referral tertiary health institution in Nigeria, receiving patients from all over the country on a daily basis. All patients recruited for this study (both males and females) were between 18 and 65 years of age. 75 patients were totally recruited for this study.

The patients were divided into two groups, 1 and 2. Group 1 consisted of patients with malignancy-related ascites and group 2-of patients with non-malignant ascites respectively.

In group 1, malignancy was documented by histology; clinical features, ultrasonography and computerized tomography (CT) scan where applicable. Patients with proven diagnosis of cancer who had negative ascitic cytology were also included in group 1.

Group 2 consist of patients with ascites from any other cause outside malignancy.

Patients diagnosed with cancer who had previously received anticancer treatment were excluded from this study.

2.1 Ethical Consideration

Ethical Committee approval was obtained for the current investigation from the research and ethical committee of Lagos University Teaching Hospital and it was done in accordance with the ethical protocol of LUTH. A written and verbal consent was obtained from each participant after a detailed explanation of the procedures involved. For the patients who were illiterates, their close relation explained the procedure to them. All subjects were requested to fill a questionnaire. Information necessary on the questionnaire included: age, sex, causes of admission, history of alcohol ingestion, smoking, drug abuse, presence /absence of co-morbid risk factor such as tuberculosis.

The procedure of ascitic fluid collection was well explained to the patients. They were compliant. After explaining the procedure, the patients were well rested, placed in supine position, and inclined at 45º on bed. The skin on the left lateral side (hypochondria) was well cleaned with 70% alcohol and cotton wool. Then using a 20 ml syringe, ascitic fluid was collected by backflow pressure into the 20 ml syringe and the needle was removed and pressure applied on the area with fresh dry cotton wool. It was collected into a universal bottle.

75 patients with ascites from various etiologies underwent abdominal paracentesis in the first 24hours after admission preferably before any intervention was done.

Before analysis, ascitic fluid was centrifuged at 12000 rpm for 5 minutes at room temperature to remove cellular debris, and it was then assayed for ascitic fluid fibronectin, total protein and albumin.

Venous blood was collected at the time of ascitic fluid collection. The procedure of venepuncture was explained to the patients. Patients were seated, or lying down, well relaxed and rested. Venous blood was aspirated from the cubital fossa, after it was well cleaned with 70% alcohol and cotton wool and allowed to dry. Then a 5ml syringe was used to collect the blood sample and put it into a sample bottle. For each one of the patients, 5 ml of blood was drawn from the vein using a 5 ml syringe. The blood obtained was spun for separation of plasma from the cells. The plasma obtained was used to assay for fibronectin, total protein and albumin.

An enzyme-linked immunosorbent assay (ELISA) kit, Assaymax Human Fibronectin ELISA kit was used(ANTIBODIES-ONLINE Inc., Atlanta, GA, USA), for quantitative determination of fibronectin in plasma and ascitic fluid and read out using Emax® micro tube well reader.

Biuret method was applied for common quantification of protein in both plasma and ascitic fluid. This method was chosen because it is the most widely used, it is not cumbersome and is highly recommended by International Federation of Clinical Chemistry (IFCC) [18]. Also, only a small quantity of sample is usually required.

For Albumin assay, Bromocresol Green (BCG) Colorimetric method was used. The principle here is that albumin binds to BCG at a pH of 4.2 to form a colored compound. The blue color formed is directly proportional to the amount of albumin. This was used to estimate the plasma and ascitic fluid albumin levels.

A commercially available protein assay kit (Randox R Laboratories Ltd, UK.) was used.

2.2 Statistical Analysis

The statistics was carried out by the SPSS® version 15.0 (SPSS Inc., Chicago, IL, USA) statistical package for Windows®. The mean values, standard deviation, as well as median values and range of plasma and ascitic fluid concentrations of fibronectin, total protein, and albumin were calculated. The variance, correlation and regression analysis were employed. Receiver Operating Characteristic (ROC) curves were calculated by standard procedures. This was created by plotting the fraction of true positive rate TPR (sensitivity) against the false positive rate FPR (1-specificity). The area under the curve is a relative measure of the diagnostic test performance. By superimposing the ROC curves of different markers of malignancy, the most predictive marker can be selected. Applying cut-off limits for the determined parameters permitted classification into four categories:

(a)True positive
(b)True Negative
(c) False positive
(d) False negative
Sensitivity = a/a+d ×100%
Specificity=b/b+c ×100%
Positive predictive value= a/a+c ×100%
Negative predictive value= b/b+d ×100%
Diagnostic efficiency= (a+b)/a+b+c+d × 100%

The significance of differences of sensitivity, specificity, efficiency between various parameters was evaluated by chi-square test. A *p* value of less than 0.05 was considered statistically significant.

Comparison of clinical and biochemical characteristics between the levels of fibronectin in malignant and non-malignant ascites was performed by the chi square test for discrete variables and the student unpaired t-test for continuous variables. The student *t*-test was applied for comparison of mean values between groups. The Pearson correlation coefficient was used to correlate the studied variables. In all the tests, the level of significance was set at $P<0.05$.

3. RESULTS

The total number of patients enrolled in the current study was 75. From this total number, 25 (33.3%) were males and 50 (66.7%) females. These patients were divided into two groups, I and 2 (Table 1).

Table 1. Showing the division of patients into two groups

Type of ascites	Male	Female	Total
Malignant (Group 1)	7	30	37(49.3%)
Non-malignant (Group 2)	18	20	38(50.7%)
Total	25(33.3%)	50(66.7%)	75(100%)

Group 1 consisted of 37 patients (49.3%) of the total 75 patients with malignancy-related ascites. This was made up of 7 males and 30 females. The etiological distribution of these 37 individuals (7 males and 30 females) was: primary liver cell carcinoma, twelve (32.4%); cancer of the cervix, five (13.5%); Ovarian cancer, eleven (29.7%); cancer of the bladder, one (2.7%) Endometrial cancer, one (2.7%); seminoma, one (2.7%), Cholangiocarcinoma, one (2.7%); Renal cell carcinoma, one (2.7%); Breast cancer, three (8.1%); undifferential abdominal neoplastic infiltration, one (2.7%).Diagnosis was confirmed by a biopsy and histology. Also, clinical features were noted in those with long-standing cancer alongside by radiological investigations like ultrasound and CT scan where affordable. However, histology confirmed malignancy in all the patients.

Group 2 consisted of 38 patients (50.7%) of the total study group and was made of patients with non-malignant ascites (18 males, 20 females).The etiological distribution of these 38 individuals (18 males and 20 females) was: congestive cardiac failure, twelve (31.6%); chronic kidney disease, seven (18.4%); liver cirrhosis, seventeen (44.8%); tuberculosis, one (2.6%); and leukemia, one (2.6%). None of the patients from this group had any malignancy.

A gender difference between the two groups was also established. Group 1 had more females, while group 2 had more males (Table 1). All

these patients were Nigerians from the three major ethnic tribes: Hausa, Ibo and Yoruba (Table 2).

The mean age of the study group was 46.58±12.44 years with a range of 18 years to 65 years. Patients in group 1 were slightly older than those in group 2 (mean age ±SD: 48.43±11.13 vs. 44.79±13.51 respectively). Summary of age distribution is seen in (Table 3).

The mean blood pressure was 122.10±17.01 mmHg for systolic value and 73.34±9.97 mmHg for diastolic blood pressure. Mean body mass index (BMI) was 24.56±3.49 kg/m^2. More than half of the study population (57.3%) was traders, businessmen/women, farmers or unskilled people by profession.

Ascitic fibronectin levels of higher than 100 µg/ml (cut-off value was 73 µg/ml) were found in 18 patients with malignancy-related ascites and in none with non-malignant ascites. Ovarian cancers had the highest value of fibronectin concentration. The mean ascitic fibronectin concentration in patients with malignant ascites was 97.54±17.73 µg/ml as against 47.76±13.32 µg/ml seen in non-malignant ascites (P<0.001). The mean ascitic fibronectin concentration in both groups was 72.32±29.97 µg/ml. At a cut-off value of 73 µg/ml for ascitic fibronectin, the sensitivity was 94.6%, specificity 94.7%, positive predictive value 94.6%, negative predictive value 94.7%, and diagnostic accuracy of 94.7% (see Table 4).

The mean plasma fibronectin was 42.73±10.02 µg/ml. Patients with malignant ascites had a mean plasma fibronectin of 46.24±10.28 µg/ml, while those with non-malignant ascites of 39.32±8.58 µg/ml (P <0.05) respectively. Patients with liver cirrhosis had lower values of fibronectin when compared to other non-malignant disease entities.

The mean ascitic fluid total protein was 34.41±16.97 g/L and 71.75±7.35 g/L for plasma concentration. Sensitivity of ascitic protein was 62.7% at a cut off limit of 41.5 g/L. The mean ascitic albumin concentration was 29.6±10.2 g/L while mean plasma albumin concentration was 40.42±7.75 g/L (see Table 4). The accuracy of ascitic albumin was 50.7%. There was a positive correlation between SAAG and ascitic fibronectin (P<0.05, r = 0.34) while there was a poor correlation between serum ascites albumin gradient (SAAG) and plasma fibronectin (Figs. 1 and 2).

As illustrated by the Receiver Operating Characteristic (ROC) curve (Figs. 3 and 4), the differential diagnostic accuracy of ascitic fibronectin was superior to that of other parameters. This observation was confirmed when sensitivity, specificity, positive and negative predictive values were calculated. Ascitic fibronectin, SAAG, total protein and albumin at discrimination points of 73 µg/ml, 11.50 g/L, 41.5 g/L, 49 g/L separated patients with malignancy from patients with non-malignant ascites with accuracy of 94.7%, 73.3%, 62.7%, 50.7% respectively (Table 5).

Table 2. Table showing distribution of ascites by tribes

Tribe	Non-malignant ascites	Malignant ascites	Total	Percentage (%)
Yoruba	21	15	36	48
Hausa	3	2	5	7
Ibo	9	11	20	27
Others	10	4	14	18
	43	32	**75**	100

Table 3. Age distribution of subjects with malignant and non-malignant ascites

Age (years)	Group 1 (Malignant) (%)	Group 2 (Non-malignant) (%)
<20	0	5.3
20-29	5.4	13.2
30-39	18.9	10.5
40-49	18.9	23.7
50-59	32.4	28.9
60-65	24.3	18.4

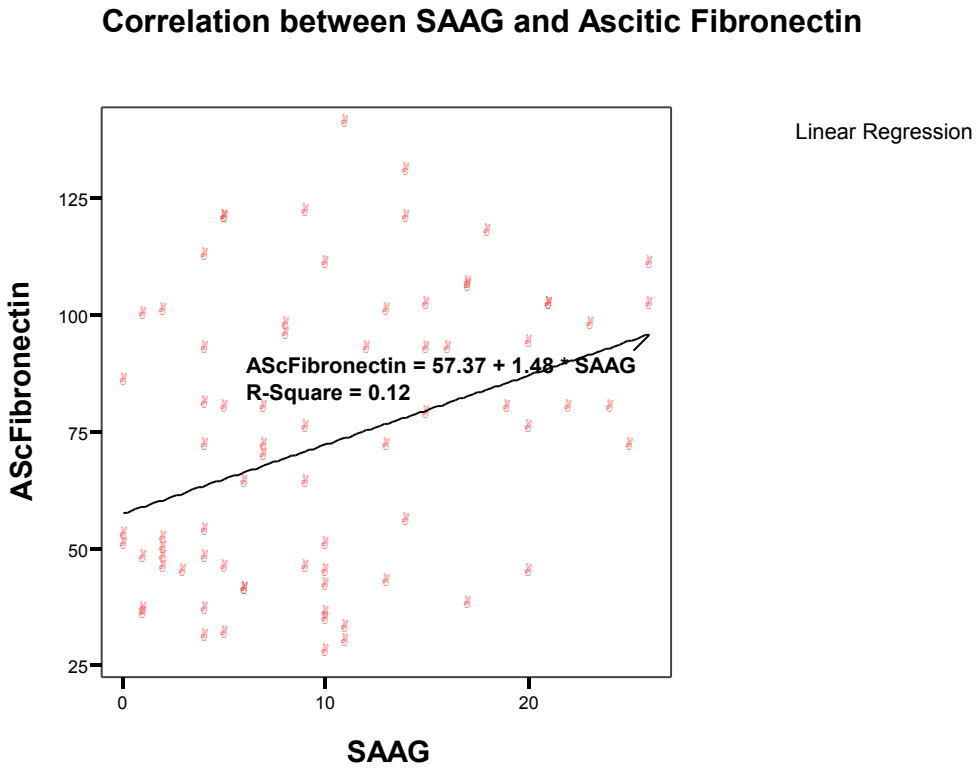

Fig. 1. Positive significant correlation between ascitic fibronectin and SAAG

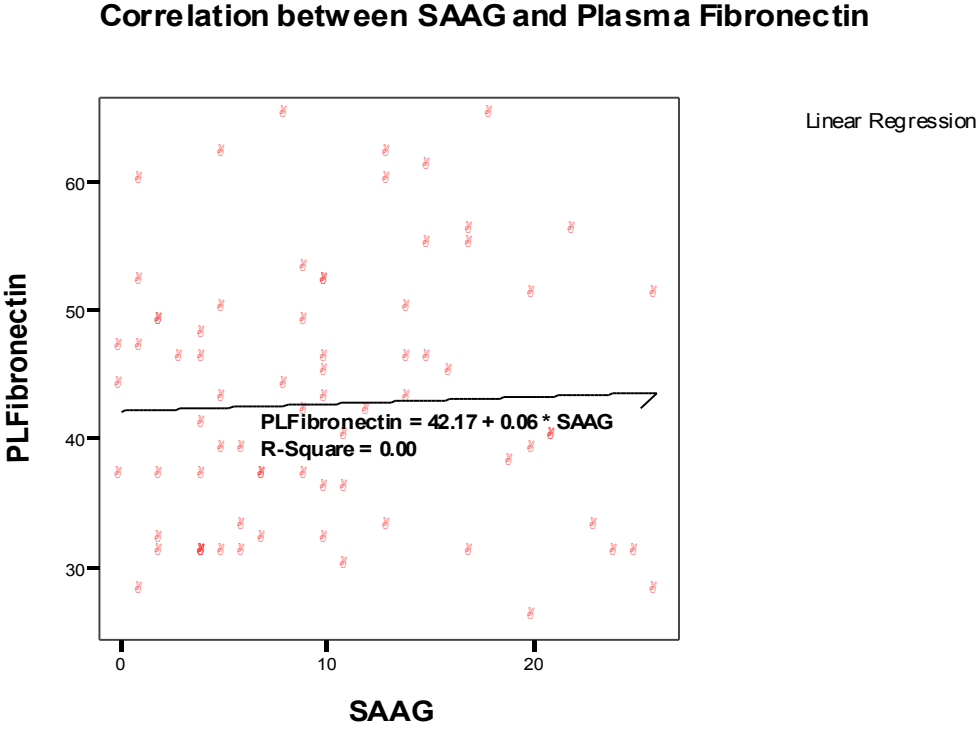

Fig. 2. Positive insignificant correlation between plasma fibronectin and SAAG

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

Parameter	NMA(x±SD)	MA(x±SD)	(Difference) P value
Fibronectin (µg/ml)	47.76±15.32	97.54±17.73	P<0.05
Total protein (g/L)	30.21±15.00	38.72±18.00	Not significant
Albumin (g/L)	31.23±10.01	28.08±10.32	Not significant
SAAG (g/L)	6.74±4.8	13.56±7.5	P<0.05

Table 5. 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)

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive predictive value (%)	Negative predictive value (%)	Cut-off value
Fibronectin	94.6	94.7	94.7	94.6	94.7	73 µg/ml
LDH	97.3	84.2	90.7	85.7	97.0	310 IU/l
Protein	37.8	86.8	62.7	73.7	58.9	41.5 g/L
Albumin	0.0	100	50.7	0	50.7	49 g/L
Cytology	56.8	100	78.6	100	70.4	
SAAG	59.5	86.8	73.3	81.5	68.8	11.5 g/L

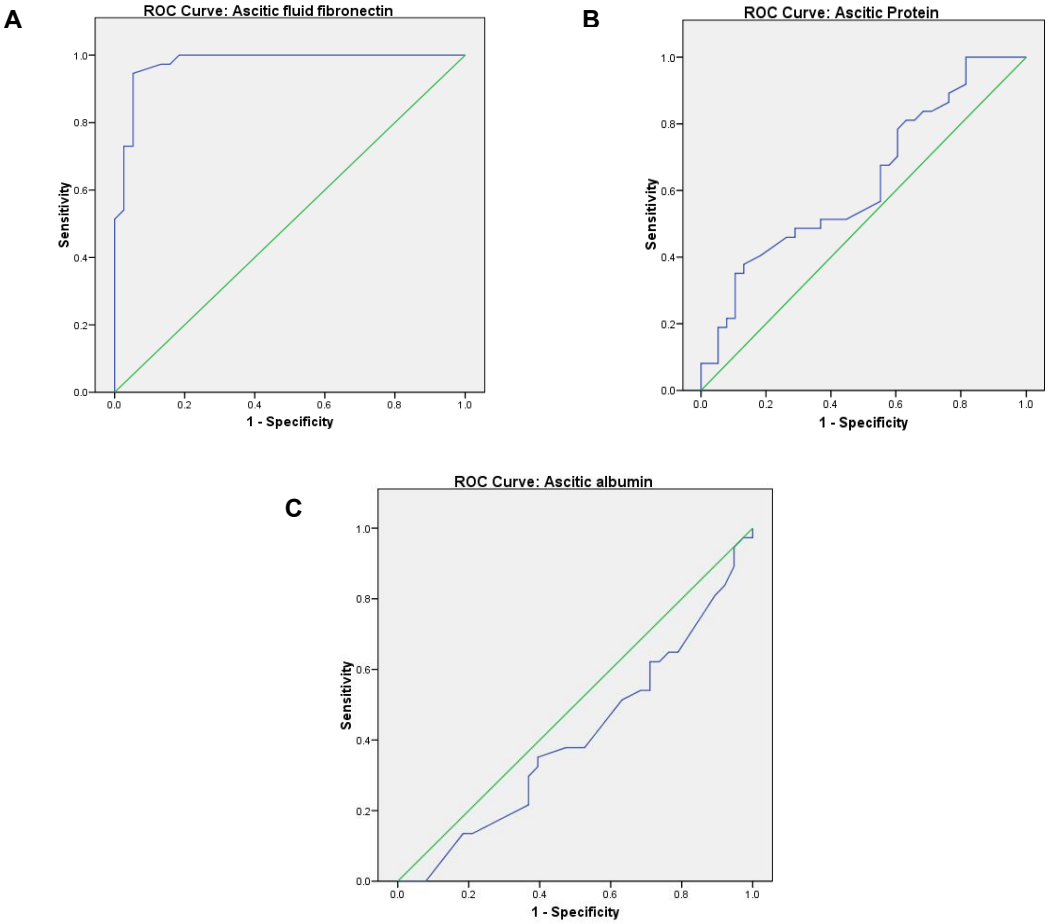

Fig. 3. ROC curve in ascitic fluid
Ascitic Fibronectin; B. Ascitic Total protein; C. Ascitic Albumin

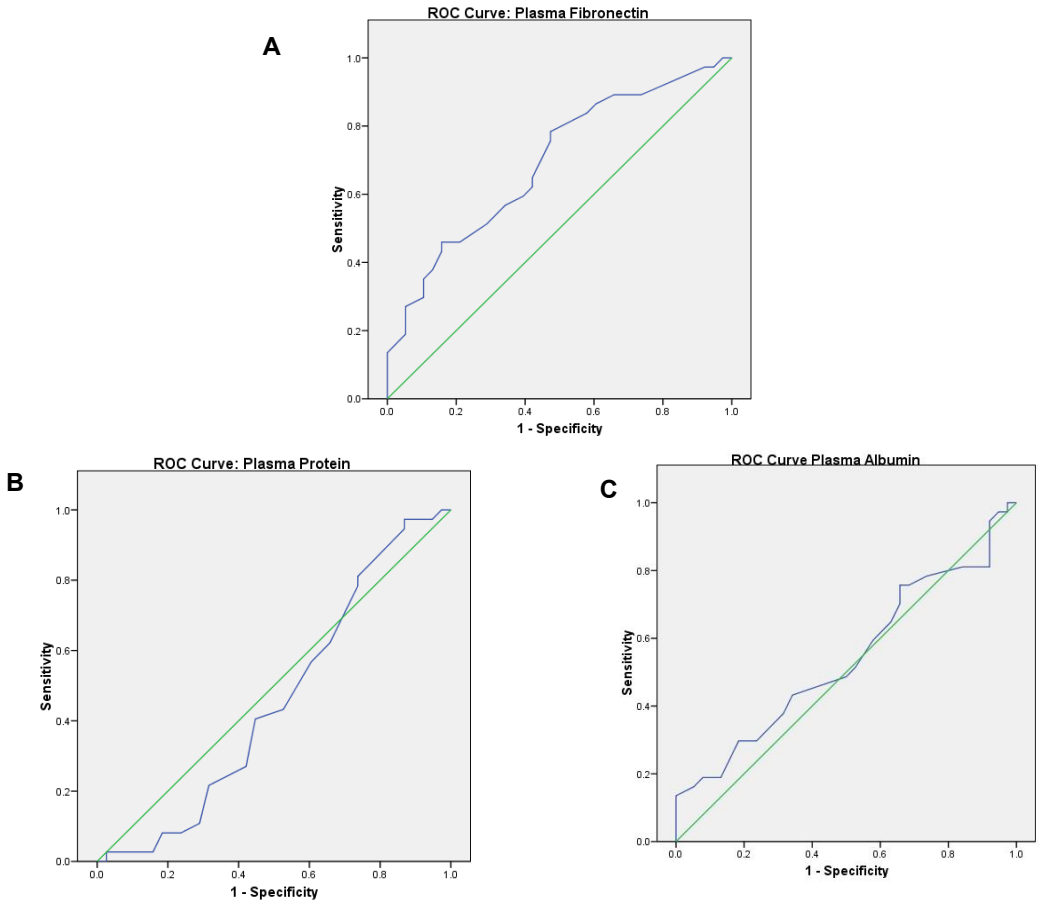

Fig. 4. ROC of analytes in plasma

A. Plasma Fibronectin; B. Plasma Total Protein; C. Plasma Albumin

4. DISCUSSION

Ascites is one of the most common clinical problems confronting physicians in Nigeria. It is a common clinical sign with a wide range of causes [3]. There are no distinctive features and no single diagnostic test is accurate in differentiating malignancy-related from non-malignant ascites [8]. Discrimination of malignancy-related ascites from non-malignant 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 [19]. The differential diagnosis of ascites remains a major clinical problem, and unfortunately so far none of the analytes used to distinguish one type from another has a diagnostic accuracy of 100%. Attempts to achieve a complete differentiation of patients with malignancy-related ascites and non-malignant ascites by means of simple laboratory test have so far failed [20].

Recent studies have drawn attention to the surface properties of cancer cells, suggesting new possible markers of malignant effusions [12]. Lipids (mainly cholesterol) and fibronectin, a high molecular weight (HMW) glycoprotein derived from the extracellular matrix have been found to be elevated in malignant ascites [8,13,21].

Cytological examination of ascitic fluid has proven rather insensitive, with detection of malignant cells in only 40-70% of malignancy-related ascites [7,9]. Castaldo et al. [22] have cited a sensitivity of 40-60% for cytology in their article. In this index study, the sensitivity of cytology was found to be 56.8%. Lack of sensitivity may be due low number of neoplastic cells present in some ascitic fluid samples [9,23-24]. As is well known, cytological examination of ascites can only detect malignancy when the tumor cells involve the peritoneum and exfoliate into the ascitic fluid

[25]. Another reason for this low sensitivity may be that most tumors shed their neoplastic cells into ascitic fluid intermittently [24].

Taking into account the limitation of cytology, ascitic fluid has been examined for other parameters which might allow for its differential diagnosis. Therefore, other parameters of ascitic fluid have been investigated for their differential diagnostic value. Some of these biochemical parameters explored in this regard so far include, ascitic fluid fibronectin, cholesterol, protein, LDH, and other tumor markers.

Recently, an accuracy of above 90% was reported for concentrations of fibronectin [23,26]. According to this index study, fibronectin levels have been shown to correlate with malignancy. Levels of fibronectin are higher in ascites associated with malignancies, whereas levels are lower in ascites related to non-malignant condition.

The most common cause of ascites between the two groups was liver cirrhosis secondary to chronic viral hepatitis (types B, and C) found in 17 patients (22.6%) of the total group investigated. This corroborates with the study done by Malabau et al. [27] at Ibadan.

Sood et al. [28] showed that high concentrations of ascitic fibronectin were significantly higher in malignancy-associated than in non-malignant ascites. They also showed that there is a link between malignancy and fibronectin levels. In this index study, the diagnostic accuracy of fibronectin in ascitic fluid was found to be 94.7%, using a cut-off value of 73 µg/ml. The sensitivity, specificity, and accuracy of fibronectin were 94.6%, 94.7% and 94.7% respectively. This agrees with earlier studies done by Sood et al. [25] who got an accuracy of 97.1% in their study and a sensitivity of 100%. Chilan JM et al. [29] showed that the diagnostic accuracy of fibronectin in differentiating malignant and non-malignant ascites was 85%. Lee CM et al. [30] also conducted similar studies and arrived at a diagnostic accuracy of 95.9% for ascitic fibronectin.

In a similar study, Siddqui et al. [31] had 100% accuracy for fibronectin as against 78.7% for malignant cytology. This implies that fibronectin may be more sensitive for diagnosis of malignant ascites. A proposal that one of the reasons for the increased levels of fibronectin in the ascitic fluid could be its increased production from the neoplastic cells, which therefore could explain the high values of this protein in malignancy-related ascites [32,33]. The transformed malignant cell may shed matrix fibronectin which then may be found in the body fluids [20]. The specificity of ascitic fibronectin in this study (94.7%), is similar to that reported by Colli et al. 8 (93%), and superior to the 88% reported by Gerbes et al. [34], but inferior to the 100% accuracy observed by Scholmerich5 and Archimandritis et al. [26] and the 98% observed by Prieto et al. [35]. It should be noted that the cut-off levels were different in the five studies mentioned.

According to the results of the current study, total protein levels were higher in malignancy-connected than in non-malignant patients, while specificity and sensitivity were low (86.8%, 37.8% respectively).These findings support the views of various investigators, that protein concentration is not a definite criterion for differentiating malignant from non-malignant ascites.

5. CONCLUSION

The presented paper focused on the usefulness of fibronectin in the differential diagnosis of ascites and these data and findings suggest that fibronectin may have potential value in oncology, it may be used as tumor marker in differentiating malignant from non-malignant ascites. Also, fibronectin is more sensitive in diagnosing malignancy, when compared to cytology. Further prospective studies on larger number of subjects might be necessary for validation of these findings.

6. LIMITATION

This study may be limited by the fact the sample study size is rather small. Using a larger number of sample size maybe needed to validate these findings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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