

Research Article**The Sensitivity of Cytology in the Differential Diagnosis of Ascites among Adult Nigerians in a Tertiary Health Institution**Ekpe EEL^{1*}, Omotoso AJ²¹Department of Chemical Pathology, University of Calabar Teaching Hospital Calabar, Nigeria²Department of Morbid Anatomy/Forensic, University of Calabar Teaching Hospital, Calabar, Nigeria***Corresponding author**

Ekpe EEL

Email: lawsonekpe2002@yahoo.com

Abstract: Ascites is a common clinical problem confronting physicians and is commonly encountered in many patients with liver diseases, cardiac failure, renal diseases, and malignancies. Currently, differentiation of the fluid by laboratory analysis into malignant and non-malignant ascites has not been fully achieved yet. The aim was to assess the diagnostic accuracy of cytology in the differential diagnosis of ascites. A total of seventy five unselected patients with ascites were studied. Thirty seven of them had malignancy-related ascites (7 males, 30 females), while 38 had non-malignant ascites (18 males, 20 females). Cytology was done for all ascitic fluid samples collected from these patients. Cytology yielded an accuracy of 78.7% and a sensitivity of 56.8%. Hence, it is found that cytology alone may not be too useful in differentiating malignant from non-malignant ascites as its sensitivity is low and so other biochemical parameters may be needed to supplement cytology in the differential diagnosis of ascites.**Keywords:** Ascites, Cytology, Malignant, Non-malignant, Differential diagnosis.

INTRODUCTION

Ascites is defined as the abnormal accumulation of fluid in the peritoneal cavity. It has been traditionally classified into exudate and transudate. This classification is based on the protein content of the ascitic fluid. However, this has now been superseded by the serum ascites albumin gradient (SAAG) which is obtained by subtracting the ascitic fluid albumin from the serum albumin [1, 2]. The causes of ascites are many. The commonest cause of ascites is liver cirrhosis [3], which accounts for about 81% (alcohol 65%, viral 10%, others 6%) of all cases of ascites. Other causes are cancer, 10%; congestive cardiac failure, 3%; tuberculosis, 2%; dialysis, 1%; pancreatic disease, 1%; and miscellaneous, 2% [4].

The exact mechanism regarding the formation of ascites remains controversial [5, 6], even though many theories try to explain the pathogenesis. Making a differential diagnoses between malignant and non-malignant ascites is very vital [7] to the management of patients. Malignancy accounts for approximately about 10% of cases of ascites [8]. Malignant diseases can cause ascites by various mechanisms [9]. Many times, the evaluation of malignancy-related ascites is based on clinical history, ascites fluid analysis and imaging tests [10].

It is a common knowledge for many years that cytology is a commonly requested investigation for many cases of ascites or metastasis to the abdominal organs presenting with ascites. This prompted our interest in assessing the diagnostic accuracy of cytology. This also prompted a literature research with a view to determining its usefulness in management of patients with ascites.

METHODOLOGY**Study population**

This was a cross sectional study done in Lagos University Teaching Hospital (LUTH) between August 2011 and July 2013. Ethical clearance was obtained from the health research and ethics committee of LUTH (REF:ADM/DCT/HREC/VOL.X VI/101). Adult unselected male and female patients presenting with obvious ascites were recruited for this study. A total of 75 consecutive patients admitted with ascites from various etiologies were recruited. The study criteria included Nigerians from various tribes aged between 18-65 years. The study was done in accordance with the hospital policy. Informed consent was sought from all patients. The patients were recruited from various departments viz: gastroenterology clinic, surgery and obstetric/gynecological clinic. The patients had abdominal paracentesis done prior to any medical or surgical management. For each patient, ascitic fluid was collected by abdominal paracentesis following

guidelines given by the American Association for Liver Diseases (AASLD)⁷. After emptying the bladder and confirming the ascites by physical examination, patients were rested and the procedure of ascitic fluid collection was well explained to the patients that were compliant with the research. Under aseptic conditions, abdominal paracentesis was performed. The ascitic fluid was collected into a universal bottle. Seventy five patients with confirmed ascites from various etiologies underwent abdominal paracentesis in the first 24 hours after admission preferably before any medical/ surgical intervention. The ascitic fluid was then centrifuged at 10,000rpm for 5mins at room temperature to separate cellular debris from the fluid. The supernatant was collected.

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 two 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. Slides examined under the microscope, if positive for malignancy showed the presence of malignant cells of various sizes, with features such as 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, but some had lymphocytes, reactive mesothelial cells and some were acellular. 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.

Group I consisted of 37 patients with malignancy – related ascites. This was made up of 7 males and 30 females. The aetiological distribution of these 37 patients 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%); intra-abdominal malignancy, one (2.7%). Clinical

features were noted in those with long-standing cancer alongside with radiological investigations like ultrasound and CT scan where affordable. However, histology confirmed malignancy in all the patients.

Group II consisted of 38 patients and was made of patients with non-malignant ascites (18 males, 20 females). Aetiological distribution of these patients were: congestive cardiac failure, twelve (31.6%); chronic kidney disease, seven (18.4%); liver cirrhosis, seventeen (44.8%); tuberculosis, one (2.6%); and lymphoproliferative disease, one (2.6%). None of the group II patients had any malignancy. This was the control group.

Data analysis

The data was input into Microsoft excel and analysis carried out using SPSS 15.0 version and *p* value of less than 0.05 was taken as significant.

RESULTS

A total of 75 adult patients were recruited ranging between 18 and 65 years. The mean age was 46.58±12.44 years. Out of the 75 people, 25(33.3%) were male, while 50(66.7%) were females. The people were drawn from all major tribes in Nigeria. The Yoruba tribe had the highest number of patients with ascites (48%).

This is possibly explained by the fact that the study was conducted in a Yoruba dominated environment. Other tribes were represented. Out of the 75 people, there was a division into two groups: Group I and Group II.

Group I was made up 37 patients which was made up of 7 males (18.9%), and 30 females (81.1%) and had the patients with malignancy-related ascites. Histology, clinical features, autopsy and radiological investigations confirmed malignancy in all patients in this group. But out of these, cytology was positive in 21 of the total 37 people in this group (56.8%). Sixteen were negative for malignancy (43.2%).

For the cytological examination, each slide was prepared and read twice by a consultant cytopathologist. A positive cytology was confirmed by the presence of pleomorphic cells, with raised nucleocytoplasmic ratio, irregular membrane borders and coarse clumped chromatin. A total of 21 cases were positive for malignancy (56.8%), while 16 were negative (43.2%). (This was for those in group I which had malignancy-related ascites). For those in group II (control group) there were no malignant cells detected in any sample. All yielded negative. There was no false positive. This gave a sensitivity of 56.8%. The specificity was 100%, while positive predictive value (PPV) and negative predictive value (NPV) were 100% and 70.4% respectively. The accuracy was given as 78.7%.

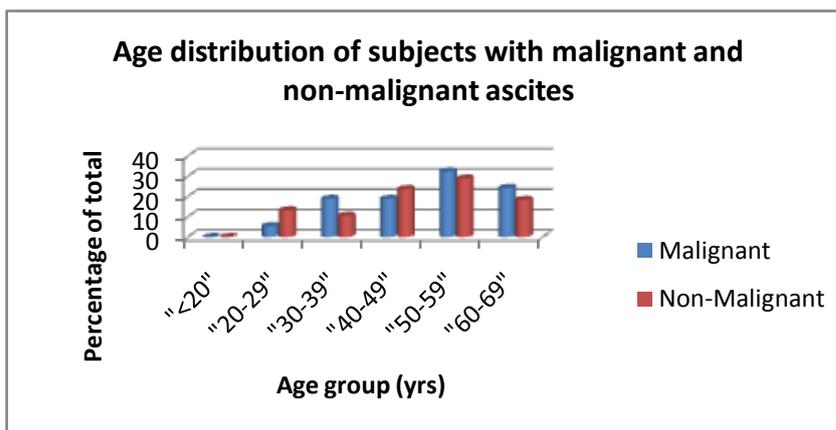


Fig. 1: Age distribution of subjects with malignant and non-malignant ascites

Table 1: Various Nigerian tribes and their distribution of ascites

Group	Control	Malignant	Total	Percentage (%)
Yoruba	21	15	36	48
Hausa	3	2	5	7
Igbo	9	11	20	27
Others	10	4	14	18
Total			100	

DISCUSSION

Ascites is a common finding among patients globally and usually the diagnostic challenge it poses cuts across all specialties of medical practice.

It is important to distinguish malignancy-related ascites from non-malignant ascites since their management modalities are not the same [11]. It is even worse to assume a malignancy is absent, when it is actually present in a patient simply due to poor diagnostic ability of cytology (sensitivity obtained here was 56.8%).

Since about 10% of all ascites are of malignant origin [8], there is need for accurate diagnosis to be made because of the metastatic effect of malignancies. Accurate and early diagnosis would go long way in forestalling the complications associated with malignancies. Too much hope is placed on cytology to provide the diagnosis at the expense of other investigations [2].

However cytologic investigation of ascitic fluid is specific but not very sensitive (40-70%) and may thus give rise to false negative values [12]. Lack of sensitivity may be due to low number of neoplastic cells in some ascitic fluid sample [13]. Cytologic examination of ascitic fluid can only detect malignancy when the tumor cells involve the peritoneum and exfoliate into the ascitic fluid [14]. Another reason for this low sensitivity may be that most tumors shed their neoplastic cells into ascitic fluid intermittently [15]. In our study, cytology had a diagnostic specificity of 100% but identified only 56.8% of malignant ascites. This agrees with the findings of other researchers [16-

19]. However, accuracy, PPV, NPV of cytology was 78.7%, 100%, 70.4% respectively.

Sensitivity depends on the type of malignancy and site of effusion. Reports from different workers suggest a high rate of cancer cell detection in carcinoma than lymphomas [20-22]. But there is yet no universal agreement as to which site gives a higher yield, the pleural or peritoneal cavity [21-23].

Cytology examination has found to be more successful in patients with peritoneal carcinomatosis as viable malignant cells are exfoliated into the ascitic fluid [10] and sensitivity of cytology in detecting peritoneal carcinomatosis is as high as 96.7% [24]. However, only 53% of patients with malignancy-related ascites have peritoneal carcinomatosis. Patients with other causes of malignancy-related ascites almost always have a negative cytology [25]. Hence, negative cytology should be interpreted with caution.

The diagnosis of cancer is confirmed only on the histologic examination of an appropriate tissue. It should however be noted that tissue diagnosis is sometimes unattainable in some instance including patients unfit to go for biopsy or surgery and on the occasions when the biopsy tissues does not adequately represent the lesion present in the organ sampled. In this case, cytology examination of the ascitic fluid may assist in clinical diagnosis of the malignancy.

In conclusion, a combination of clinical history/presentation, cytology, radiological investigation and biochemical assessment of patients

should be the mainstay of making diagnosis of malignancy and not just cytology alone.

REFERENCES

1. McHutchinson JG; Differential diagnosis of ascites. *Sem Liver Dis.*, 1997; 17(3): 191–201
2. Karoo ROS, Lloyd TDR, Garcea G, Redway HD, Robertson GSR; How valuable is ascitic cytology in the detection and management of malignancy. *Postgraduate Medical Journal*, 2003; 79(931): 292-294.
3. American College of Gastroenterology; Ascites: A common problem in people with cirrhosis. Available from <http://patients.gi.org/topics/ascites/>
4. Ascites. Available from <http://enotes.tripod.com/ascites.htm>
5. Wilkinson SP, Moore KP, Arroyo V; Pathogenesis of ascites and hepatorenal syndrome. *Gut Supplement*, 1991, S 12-S 17. Available from <http://gut.bmj.com/content/32/supplement/S12.full.pdf>
6. Wong F, Girgrah N, Blendis L; Review: the controversy over the pathophysiology of ascites formation in cirrhosis. *J Gastroenterol Hepatol.*, 1997; 12(6): 437-444.
7. Tuzun Y, Yilmaz S, Dursun M, Canoruc F, Celik Y, Cil T *et al.*; How to increase the diagnostic value of malignancy-related ascites: discriminative ability of the ascitic tumour markers. *J Int Med Res.*, 2009; 37(1): 87-95.
8. Liu F, Kong X, Dou Q, Ye J, Xu D, Shang H *et al.*; Evaluation of the tumor markers for the differential diagnosis of benign and malignant ascites. *Annals of Hepatology*, 2014; 13(3): 357-363.
9. Runyon BA, Hoefs JC, Morgan TR; Ascitic fluid analysis in malignancy-related ascites. *Hepatology*, 1988; 8(5): 1104-1109.
10. Mayo Clinic; Test 61530: Carbohydrate Antigen 19-9 (CA 19-9), Peritoneal Fluid. Available from http://www.mayomedicallaboratories.com/interpretive-guide/?alpha=C&unit_code=61530
11. Sangisetty SL, Miner TJ; Malignant ascites: A review of prognostic factors, pathophysiology and therapeutic measures. *World J Gastrointest Surg.*, 2012; 4(4): 87–95.
12. Castaldo G, Oriani G, Cimino L, Topa M, Mostarda I, Castellano L *et al.*; Total discrimination of peritoneal malignant ascites by assay of ascitic cholesterol and lactate dehydrogenase. *Clin Chem.*, 1994; 40(3): 478-483
13. Banerjee M, Singh R, Arora MM, Srinivas V, Basannar D, Patrikar S; Biomarkers of malignant ascites—a myth or reality. *MJAFI*, 2011; 67(2): 108-112.
14. Feldman M, Friedman LS, Brandt LJ; Sleisenger and Fordtran's *Gastrointestinal and liver disease: Pathophysiology, diagnosis, management.* Elsevier Health Sciences, 2015: 1562.
15. Prieto M, Gómez-Lechón MJ, Hoyos M, Castell JV, Carrasco D, Berenguer J; Diagnosis of malignant ascites: comparison of ascitic fibronectin, cholesterol and serum ascites albumin difference. *Dig Dis Sci.*, 1988; 33(7): 833 – 838.
16. Motherby H1, Nadjari B, Friegel P, Kohaus J, Ramp U, Böcking A; Diagnostic accuracy of effusion cytology. *Diagn Cytopathol.*, 1999; 20(6): 350-357.
17. Jha R, Shrestha HG, Sayami G, Pradhan SB; Study of effusion cytology in patients with simultaneous malignancy and ascites. *Kathmandu Univ Med J.*, 2006; 4(4): 483-487.
18. Gerbes AL, Jungst D, Xie YN, Permanetter W, Paumgartner G; Ascitic fluid analysis for the differentiation of malignancy-related and non-malignant ascites. Proposal of diagnostic sequence. *Cancer*, 1991; 68(8):1808-1814.
19. Sari R, Yildirim B, Sevinc A, Bahceci F, Hilmioglu F; The importance of serum and ascites fluid alpha-fetoprotein, carcinoembryonic antigen, CA19-9, and CA 15-3 levels in differential diagnosis of ascites etiology. *Hepatogastroenterology*, 2001; 48(42): 1616-1621.
20. Luse SA, Reagan JW; A histocytological study of effusions: Part II: Effusions associated with malignant tumors. *Cancer*, 1954; 7(6): 1167-1181.
21. Melamed MR; The cytological presentation of malignant lymphomas and related diseases in effusions. *Cancer*, 1963; 16(4): 413-431.
22. Junaid TA, Odor EIA; Cytologic diagnosis of ascitic fluid in Ibadan, Nigeria. *Journal of the National Medical Association*, 1980; 72(7): 669-672.
23. Naylor B, Schmidt RW; The case for exfoliative cytology of serous effusions. *Lancet*, 1964; 1(7335): 711-712.
24. Runyon BA, Hoefs JC, Morgan TR; Ascitic fluid analysis in malignancy-related ascites. *Hepatology*, 1988; 8(5): 1104–1109.
25. Mayo Clinic; Test 61528: Carcinoembryonic Antigen (CEA), peritoneal fluid. Available from http://www.mayomedicallaboratories.com/interpretive-guide/?alpha=C&unit_code=61528