The Relevance of Ascitic Lactate Dehydrogenase (LDH) and Serum Ascites Albumin Gradient (SAAG) in the Differential Diagnosis of Ascites among Patients in a Nigerian Hospital

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Authors’ contributions

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

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ABSTRACT

Aim: The study aimed to determine the diagnostic values for lactate dehydrogenase (LDH) and serum ascites albumin gradient (SAAG) with maximum sensitivity and minimum false positivity so as to differentiate malignancy-related ascites from non-malignant ascites in South West region of Nigeria.

Study Design: This is a cross sectional study to determine the correlation between ascitic fluid LDH and SAAG 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.

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INTRODUCTION

Ascites is the pathological accumulation of fluid in the peritoneal cavity. It is usually the end result of complications of various disease entities [1]. It is also a common clinical condition confronting physicians [1]. Runyon et al. [2] reported parenchymal liver disease as the most common cause of ascites in about 80% of individuals. This is followed by malignancy, 10%; congestive cardiac failure, 5%; tuberculosis, 2% and other causes make up about 3% [2,3]. Many studies have been carried out globally that centered on the differential diagnosis of ascites. The differential diagnosis of ascites is a common clinical problem [1]. Ascitic fluid studies are fundamental not only in for the effective care of the patients but also establishing a reliable diagnostic test to identify the etiology of the diseases, both benign and malignant [4]. No laboratory test is completely able to differentiate malignant ascites from ascites associated with cirrhosis [5]. The presence of ascites can be related to malignant or non-malignant diseases; the differentiation between these two conditions is of considerable clinical significance for further diagnostic and therapeutic procedures [6].

Traditionally, ascites has been classified as being either exudates or transudate based on ascitic fluid protein concentration or upon ascitic fluid to serum ratio of total protein or lactate dehydrogenase (LDH) or upon the ascitic fluid level of LDH [7,8]. It can also be classified based on the specific gravity.

The effective way of making the differential diagnosis of ascites is the analysis of ascitic fluid for various parameters. Some of these common parameters used in the differential diagnosis of ascitic fluid are LDH [9], albumin [10], amylase [9,11], total protein [12-14] and cholesterol [13,15]. Since there are no distinct clinical features to distinguish between malignant and non-malignant ascites, there is need for full exploration of the available biochemical parameters to enhance the differential diagnosis of ascitic fluid.

The present study was done to differentiate malignant and non-malignant ascites by use of LDH and albumin. Therefore, we determined the levels of lactate dehydrogenase and serum ascites albumin gradient (SAAG) in differentiating malignant from non-malignant ascites.

METHODS

A total of 75 patients with ascites admitted into Lagos University Teaching Hospital (LUTH) from 2011 to 2013 were enrolled for the study. Thirty seven (7 males, 30 females) had malignancy-related ascites while 38(18 males, 20 females) had non-malignant ascites. Levels of LDH and SAAG were determined in all patients with ascites. Statistical analysis was performed using SPSS software application (version 15.0) and p<0.05 was considered statistically significant and results expressed as mean ± standard deviation.

RESULTS

A total of 75 patients were recruited for the study. Twenty-five of them (33.3%) were males while 50(66.7%) were females. The mean age for both sexes was 59.03±13.54 years. Using Receiver Operator Characteristic (ROC) curve, cut-off levels were 11.5 for SAAG and 310 IU/I for LDH. These cut-offs divided the malignant from the non-malignant group. Higher levels of ascitic LDH were seen in the malignant group (900.67±918.45 IU/I) when compared to the non-malignant group (199.29±194.53 IU/I). This was statistically significant (P<0.05). The diagnostic accuracy of LDH was 90.7%. SAAG was lower in the malignant (6.74±4.84 g/L) group when compared to the non-malignant (13.56±7.50 g/L). This was also statistically significant (P<0.05). The diagnostic accuracy of SAAG was 73.3%.

CONCLUSION

It was concluded that measurement of ascitic fluid LDH and SAAG were relevant in differentiating malignant from non-malignant ascites. The determined cut-off values for LDH and SAAG in this study provides the distinctive differential diagnosis between malignant and non-malignant ascites. Routine analysis of serum and ascitic fluid albumin and LDH will resolve the problem of malignant and non-malignant ascites especially in low-resource areas or in the developing world.

Keywords: Lactate dehydrogenase; malignant; albumin; ascites; non-malignant; fluid.
male and female non-selected subjects who presented with obvious ascites were recruited for this study. A total of 75 consecutive patients admitted with ascites from various etiologies were recruited for the study. All the recruited patients were aged between 18-65 years as emphasis was on adult patients with ascites. The study was done in accordance with the hospital policy. Informed consent (both written and verbal) was sought from all patients and they were recruited from gastroenterology clinic, surgery and obstetric/gynecological clinic.

For each of the patient, about 5 mls of venous blood was collected by venepuncture at the same time of collecting the ascitic fluid by abdominal paracentesis. The procedure of venepuncture was explained to the patients. Patients were seated, or lying down, well relaxed and rested. The skin on the cubital fossa was well cleaned with 70% alcohol and cotton wool and allowed to dry. Then a 5 ml syringe was used to collect the blood sample and put into a sample bottle container. Plasma was separated from cells by centrifugation at 12,000 rpm at room temperature for 5 minutes. This separated the blood cells from the plasma. The plasma obtained was used to assay albumin, and lactate dehydrogenase. Bromocresol Green (BCG) colorimetric method was used for albumin assay. This was done by commercially available assay kits (Randox®Laboratories Ltd, UK). The levels of ascitic fluid and plasma lactate dehydrogenase were estimated by automated Hitachi machine. The catalytic activity of LDH was determined by the decrease in absorbance at 340nm. Samples for ascitic fluid and plasma LDH were assayed immediately.

The patients had abdominal paracentesis done before any therapeutic management. For each patient, ascitic fluid was collected by abdominal paracentesis following guidelines given by the American Association for Liver Diseases (AASLD) [16]. 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. Under aseptic conditions, abdominal paracentesis was performed. They were placed on supine position and inclined at 45° on the bed. The skin on the left lower quadrant of the abdomen was well cleaned with 70% alcohol and cotton wool and allowed to dry. Then using a 22 gauge needle and a 20 ml syringe, ascitic fluid was collected by a backflow pressure into the 20ml syringe and the needle was removed and pressure applied on the area with a fresh cotton wool. 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 10,000 rpm for 5 minutes 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 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 (see figures 7 and 8). 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 clinical history/details, signs and symptoms, ultrasound scan including CT scan if available, histology of the tissue/organ affected by the cancer) to assess the sensitivity of cytology in detecting malignancy.

2.1 Data Analysis

The data was input into Microsoft excel and analyzed using the SPSS® version 15.0 (SPSS Inc., Chicago, IL, USA) statistical package for Windows®. Students t-test was used and alongside with Pearson’s correlation. P values of less than 0.05 and 0.001 were considered statistically significant. Receiver Operating Characteristic (ROC) curves was 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 lactate dehydrogenase classification into four categories:

(a) True positive
(b) True Negative
(c) False positive
(d) False negative.

3. RESULTS

3.1 Distribution of Patients

The total number of patients enrolled in this study was 75. Of this total, 25(33.3%) were males and 50(66.7%) were females (Table 1).

A clear diagnosis of malignancy was made based on a combination of clinical history and presentation, biopsy for histology, radiological (computed tomography/abdominal scan) or autopsy. Based on this, the patients were divided into two groups 1 and 2.

Group 1 consisted of 37 patients with malignancy – related ascites. This was made up of 7 males and 30 females. The etiological 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%); abdominal malignancy, one (2.7%). Diagnosis was confirmed by a biopsy and histology. Also, 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 2 consisted of 38 patients and was made of patients with non-malignant ascites (18 males, 20 females). Etiological distributions 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 leukemia, one (2.6%). None of the group 2 patients had any malignancy.

LDH levels in ascitic fluid were obviously higher in the malignant group than in non-malignant group (mean value of 900.67±918.45 IU/l vs. 199.29±194.53 IU/l respectively) (p value <0.05). The same applies to the plasma LDH (mean values of 1073.15±458.89 IU/l for malignant group compared to 350.29±244.27 IU/l in non-malignant group (p<0.05). The mean plasma LDH for both groups was 706.89±514.44 IU/l, while the mean ascitic fluid LDH was 545.31±744.26 IU/L. The diagnostic accuracy of LDH was 90.7% at a cut off limit of 310 IU/L.

The mean SAAG was 10.11±7.13 g/l for the study population which was observed to be 13.56±7.50 g/l for the non-malignant group and 6.74±4.84 g/L for the malignant group (P<0.05). (Tables 2, 3 and 4). The accuracy of SAAG was 73.3% at a cut off limit of 11.50 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. Accuracy of ascitic albumin was 50.7%.

As illustrated by the Receiver Operating Characteristic (ROC) curves (Figs. 1, 2, 3, and 5), the differential diagnostic accuracy of ascitic LDH was superior to that of other parameters. This observation was confirmed when sensitivity, specificity, positive and negative predictive values were calculated. The ROC curve for ascitic albumin did not prove any appreciable sensitivity (see figure 4). Ascitic fluid LDH, SAAG and albumin at discrimination points of 310 IU/L, 11.5 g/L, 49 g/L separated patients with malignancy from patients with non-malignant ascites with accuracy of 90.7%, 73.3%, 50.7% respectively. Malignancy was most common in adults between 50-59 years (Fig. 6) and some were picked by cytology (Fig. 7).

<table>
<thead>
<tr>
<th>Type of ascites</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant (group 1)</td>
<td>7</td>
<td>30</td>
<td>37(49.3%)</td>
</tr>
<tr>
<td>Non-malignant (group 2)</td>
<td>18</td>
<td>20</td>
<td>38(50.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>25(33.3%)</td>
<td>50(66.7%)</td>
<td>75(100%)</td>
</tr>
</tbody>
</table>
Table 2. The mean, median and range of the variables of ascitic fluid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (malignant)</th>
<th>Group 2 (non-malignant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (IU/L)</td>
<td>900.60±918.45</td>
<td>199.29±194.53</td>
</tr>
<tr>
<td>Median</td>
<td>700.0</td>
<td>122.5</td>
</tr>
<tr>
<td>Range</td>
<td>200-600</td>
<td>10-1000</td>
</tr>
<tr>
<td>SAAG (g/L)</td>
<td>6.74</td>
<td>13.56</td>
</tr>
<tr>
<td>Median</td>
<td>14.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Range</td>
<td>0-20</td>
<td>0-26</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>31.23±10.01</td>
<td>28.08±10.32</td>
</tr>
<tr>
<td>Median</td>
<td>31.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Range</td>
<td>9-48</td>
<td>7-42</td>
</tr>
</tbody>
</table>

Table 3. Results of analysis of malignant and non-malignant ascitic fluid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NMA (x±SD)</th>
<th>MA (x±SD)</th>
<th>(Difference)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH (IU/L)</td>
<td>199.29±194.53</td>
<td>900.67±918.49</td>
<td>P&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>31.23±10.01</td>
<td>28.08±10.32</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>SAAG (g/L)</td>
<td>13.56±7.50</td>
<td>6.74±4.84</td>
<td>P&lt;0.05*</td>
<td></td>
</tr>
</tbody>
</table>

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

Table 4. Receiver operating characteristic (ROC) curve analysis of ascitic fluid variables

<table>
<thead>
<tr>
<th>Ascitic fluid analyte</th>
<th>Area under the curve (AUC)</th>
<th>p –value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>0.951</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.406</td>
<td>P=0.163</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Lactate Dehydrogenase is widely distributed in many body tissues. It is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD+ as a hydrogen acceptor. Patients with malignant diseases show increased LDH activity in serum; up to 70% of patients with liver metastases and 20% to 60% of patients with other nonhepatic metastases have elevated total LDH activity. The ascitic LDH levels of the malignant group were significantly higher than those of non-malignant group. The specificity and sensitivity of LDH were 84.2% and 97.3% respectively. This finding is an agreement with other researchers.
Ascitic fluid LDH is therefore a better index for screening of malignancy as a cause of ascites compared to cytology, which has a sensitivity of 40-70% [17]. Boyer et al. [7] have published a mean ascitic fluid LDH of 913±228 IU/L in the malignant group. In this study, the mean ascitic LDH obtained was 1073±458 IU/L which agrees with the work of Boyer et al. [7]. It is thought that, LDH being a non-specific tumor marker, its levels increases during malignancy. This might explain why the levels of LDH are higher in the ascitic patients with malignancy than in the non-malignant group [9].

Fig. 3. ROC of plasma LDH

Fig. 4. ROC of plasma albumin

Fig. 5. ROC curve for SAAG
Fig. 6. Ages of patients in relation to the presence of ascites depicting the age range with highest incidence of ascites

Slide showing malignant ascites

Fig. 7. Slide showing malignant cells arranged in irregular clusters (long arrow). The malignant cells are round with dark nuclei, pronounced nucleoli and pale vacuolated cytoplasm. The background shows reactive mesothelial cells (arrow head)
Fig. 8. Slide showing ascitic fluid cytology of benign origin

SAAG has been studied to verify its usefulness in the differentiation of ascites into malignant and non-malignant ascites. It is known that SAAG is a parameter of oncotic pressure gradient reflecting presence or absence of portal hypertension [10]. SAAG ≥ 1.1 g/dl is indicative of portal hypertension with an accuracy of about 97% [17].

Though SAAG does not explain the pathogenesis of ascites formation; neither is it sensitive in the diagnosis of malignancy-related ascites [18]. Lee et al. [17] concluded that SAAG offered a sensitivity of 100% and a accuracy of 96.8%. In our study, the accuracy obtained was 73.3%. The SAAG was lower in malignant ascites than in non-malignant ascites. This agrees with studies done by other researchers [10, 18]. This study also shows that ascitic total protein levels were lower in malignant patients than in non-malignant patients, while specificity and sensitivity were low (86.8%, 37.8% respectively).

However, protein concentration is not a definite criterion for differentiating malignant from non-malignant ascites. Though, mean ascitic fluid total protein is a useful marker to differentiate the main causes of ascites in patients with high SAAG [19].

5. CONCLUSION

Determining levels of LDH and SAAG in ascitic fluid is highly relevant in the differential diagnosis of ascites. Routine analysis of serum and ascitic fluid albumin and LDH will resolve the problem of malignant and non-malignant ascites especially in a low-resource centre or in the developing world.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


