Fluid Electrophoresis as a Tool for Diagnosis in Ascitic Patients: A Cross-sectional Study

YASSIR OA SIDAHMED[1], AIMUN AE AHMED[2], REHAB OE ELHEWAIG[3], YASIR S KALODA[4]

ABSTRACT

Introduction: Ascites is a condition in which there is accumulation of pathological fluid within the abdominal cavity. It is a challenging problem for healthcare personnel due to different pathological causes. To make a differential diagnosis, many investigations are needed. These investigations mainly depend on the analysis of ascitic fluids.

Aim: To determine electrophoresis patterns in ascitic fluid and to relate the findings to each established diagnosis.

Materials and Methods: This was a cross-sectional hospital-based study conducted at Khartoum and Alshaab Teaching Hospitals in Khartoum state, Sudan, from May 2018 to August 2018. The study population included all patients who were confirmed to have ascites using ultrasound. Fresh ascitic fluid samples were obtained from a total of 35 patients. All safety and aseptic conditions were applied. Immediately after collection, the samples were brought to the laboratory for electrophoresis of ascitic fluids. Data were analysed using descriptive statistics in terms of frequency and percentage.

Results: The study findings revealed that most of the patients were men 26 (74.3%) and were over 50 years old 16 (45.7%). The majority of them were diagnosed with Liver Cirrhosis (LC) (28.6%), while a few (5.7%) suffered from Periportal Fibrosis (PF), Hepatocarcinoma (HC), Ovarian Carcinoma (OC), Heart Failure (HF), or Peritonitis (PT). Different patterns of ascitic electrophoresis were detected in diagnosed patients with ascites. About 29 (82.9%) patients had specific electrophoresis patterns that helped with diagnosis, while only 6 (17.1%) did not.

Conclusion: Liver cirrhosis, hepatocellular carcinoma, and hepatitis B bands were specific compared to normal serum patterns, while no changes in ascitic fluid electrophoresis were observed in HF.

Keywords: Abdominal cavity, Ascitic fluid, Diagnosis, Liver cirrhosis

INTRODUCTION

Ascites describes the condition of the accumulation of pathological fluid within the abdominal cavity. Healthy men have little or no intraperitoneal fluid, while women may normally have 20 mL depending on the phase of the menstrual cycle [1]. Many aetiological factors have been documented as causative factors for ascites, including portal hypertension and hypoalbuminaemia [2], hepatocellular carcinoma [3], gastrointestinal cancer for malignant ascites [4], and portal hypertension leading to ascites include heart abnormalities, liver diseases, infections, and hypoalbuminaemia [5-8]. Other causes of ascites include Nephrotic Syndrome (NPh), ovarian disease, tuberculous peritonitis, and malignant conditions [7].

The work-up of patients with ascites includes a lot of effort such as history, physical examination, blood tests, abdominal ultrasound, abdominal paracentesis, ascitic fluid analysis, biochemical testing, non biochemical testing, and diagnostic laparoscopy to reach a definite diagnosis [9]. A study conducted by Dakwar GR et al., compared electrophoresis patterns of both human ascites fluid from peritoneal carcinomatosis patients and human serum from healthy donors to mice intraperitoneal fluid using capillary and agarose gel electrophoresis. They found a similar composition with an albumin fractures for human samples, while for mice, the major fractions were albumin and transferrin [10]. The presence or absence of specific proteins in ascitic fluid allows the distinction of specific protein electrophoresis bands in patients with various types of ascites, indicating the specificity of certain patterns [11]. The presence of specific bands correlated to already established diagnosis can suggest the detection of specific proteins and thus can be taken as a specific pattern indicator [12].

Electrophoresis in this study is suggested to be used for ascites diagnosis because it could be useful as a single diagnostic method rather than multiple investigations. In the cirrhotic pattern of serum protein electrophoresis, the distinction between beta and gamma globulin is blurred and is sometimes referred to as the "beta-gamma bridge" pattern [13]. The nephrotic pattern of serum protein electrophoresis illustrates the long-term loss of lower molecular weight proteins, for example, albumin and IgG, and the retention of higher molecular weight proteins, for example, alpha-2-macroglobulin [14]. An experiment by Mustika S et al., was carried out to evaluate the factors involved in the production of ascitic fluid and to determine the various types of fluid [15]; proteins were studied by electrophoresis and compared with proteins of normal blood serum in each case. They found that serum protein electrophoresis revealed an M spike in the gamma region.

Ascitic patients are diagnosed by clinical, imaging, and laboratory investigations, which include ascitic fluid analysis. However, this process is tedious, and currently, no ascitic fluid electrophoresis patterns are used for rapid diagnosis. The search for a new tool to overcome this medical challenge is necessary. Hence, this study was carried out to observe the ascitic electrophoresis patterns and to relate the findings with each established diagnosis.

MATERIALS AND METHODS

The present study was a cross-sectional hospital-based study conducted at Khartoum and Alshaab Teaching Hospitals in Khartoum state, Sudan, from May to August 2018. After approval of the study protocol from the Department of Biochemistry at the Faculty of Medicine of Khartoum University, informed consent was obtained from each patient. Verbal informed consent was also obtained from both the hospital administrations and the consultants of the units.

Inclusion criteria: All diagnosed cases of ascites admitted to the two selected hospitals during the study period were included in the study.

Exclusion criteria: All patients who were not clinically diagnosed and confirmed by ultrasound attending the two selected hospitals and who did not agree to give informed consent were excluded from the study.

Sample size: All available samples (n=35) during the study period were taken from inpatients, and ascitic fluid samples were collected.

Study Procedure

A standardised data collection form was used, which included patient's personal history, complaints, duration of ascites, onset, alcohol consumption, and previous and recent diagnosis. Full clinical examinations were conducted, including a general check-up for pallor, jaundice, clubbing, as well as abdominal examination for distension, organomegaly, lower limb oedema, and any other signs. A fresh ascitic fluid sample was obtained following all safety protocols and under proper aseptic conditions. Immediately after collection, the samples were brought to the laboratory for ascitic fluid analysis, including biochemical, cytology, total protein, albumin, and microbiology before electrophoresis analysis.

The electrophoresis apparatus (Paper Electrophoresis Kit Chamber Manufacturer, India) was utilised. A 100 mL of Barbiton buffer (pH 8.6-1.84 g barbituric acid, 10.3 g of its sodium salt per liter of water) was placed in the electrophoresis tank. Cellulose paper (Cellulose acetate strip) was handled with forceps and laid on clean paper. A line of 3 cm was drawn from the edge of the cellulose paper; it marked the origin for the application of the sample. The strip was moistened with the buffer by gently dropping it onto the buffer surface in the Perspex tank for five minutes. The strip was blotted on a sheet of clean filter paper to remove excess buffer. The strip was placed across the bridge, the power supply was turned on, left for five minutes to activate the cellulose acetate, and then turned off. The method described by Block RJ et al., was applied for the sample analysis [16].

The primary variables of the study were sex and age, while the secondary ones were the clinical diagnosis of patients with ascites and ascitic fluid electrophoresis patterns. Associations were made between demographic parameters, diagnosis, and the specificity of electrophoresis patterns of ascitic fluid compared to normal serum to identify any similarities. All obtained electrophoresis band patterns were compared with the band patterns of normal serum electrophoresis.

STATISTICAL ANALYSIS

The statistical evaluation was performed using the Statistical Package for the Social Sciences (SPSS) computer program version 16.0. The data were analysed using descriptive statistics in terms of frequency and percentage.
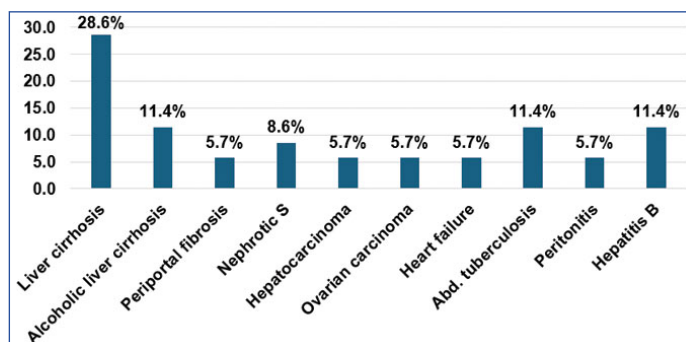
RESULTS

Most of study population were men, 26 (74.3%), and 16 (45.7%) of them were over 50 years old [Table/Fig-1].

Patterns	n (%)
Sex	
Males	26 (74.3)
Females	9 (25.7)
Age (in years)	
18-29	5 (14.3)
30-39	2 (5.7)
40-49	12 (34.3)
> 50	16 (45.7)

[Table/Fig-1]: Demographic profile and distribution of ascites patients. n=35.

Most of the patients were diagnosed with LC 10 (28.6%) [Table/Fig-2].

[Table/Fig-2]: Differential diagnosis of ascites patients included in the study, n=35.

The study findings showed that among the 10 cases diagnosed with LC 7, 20% were females and 3 (8.5%) were males. While alcoholic LC, Abdominal Tuberculosis (ATB), and hepatitis B presented 4 (11.4%) of each were male patients. The study findings showed that among the 10 cases diagnosed with LC 6 (17.1%) cases were aged 40-49 years, 3 (8.5%) cases were 18 to 29 years, and 1 (2.9%) case was 30-39 years [Table/Fig-3].

Diagnosis	Sex, N (%)		Age (in Years), N (%)			
	Males	Females	18-29	30-39	40-49	> 50
Liver Cirrhosis (LC)	3 (8.6)	7 (20)	3 (8.6)	1 (2.9)	6 (17.1)	0
Alcoholic Liver Cirrhosis (LC)	4 (11.4)	0	2 (5.7)	1 (2.9)	1 (2.9)	0
Periportal Fibrosis (PF)	2 (5.7)	0	0	0	2 (5.7)	0
Nephrotic Syndrome (NPh)	3 (8.6)	0	0	0	3 (8.6)	0
Hepatocarcinoma (HC)	2 (5.7)	0	0	0	0	2 (5.7)
Ovarian Carcinoma (OC)	0	2 (5.7)	0	0	0	2 (5.7)
Heart Failure (HF)	2 (5.7)	0	0	0	0	2 (5.7)
Abd. tuberculosis	4 (11.4)	0	0	0	0	4 (11.4)
Peritonitis (PT)	2 (5.7)	0	0	0	0	2 (5.7)
Hepatitis B	4 (11.4)	0	0	0	0	4 (11.4)

[Table/Fig-3]: Relationship between diagnosis and sex or age of the study group. n=35.

All non specific electrophoresis patterns, 6 (17.1%), were seen in patients older than 50 years [Table/Fig-4].

Applying electrophoresis to diagnose, the studied cases revealed that OC 2 (33.3%) showed non specific patterns, while ATB and PT showed a mixed pattern with various ratios [Table/Fig-4].

Ascitic fluid electrophoresis analysis of 13 cases showed the following: one case of HF, one case of PT, two cases of Alcoholic Cirrhosis (ALC), one case of HC, four cases of LC, one case of OC, one case of PF, one case of NPh, and one case of Hepatitis-B (HB) showed β- and diffuse γ-globulin bands increased in all cases, except for the patient with NPh where no bands were observed in electrophoresis. Regarding HC, it had a distribution of bands similar to normal serum but with an increase in the albumin band and diffuse gamma bands. Patients with OC showed a large increase in all bands, especially albumin and beta and gamma bands. HF showed the same band distribution compared to normal serum but appeared diluted [Table/Fig-5a,d].

Among 12 patients (one case of HB, five cases of LC, one case of NPh, one case of PF, one case of HC, one case of OC, and two cases of ATB), four cases (two ATB, HC, and OC) had increased albumin bands. Two of them showed a diffuse increase and decrease in the gamma bands [Table/Fig-5b].

Among five patients (one case of HF, two cases of LC, one case of PT, and one case of HB peritonitis), the electrophoresis bands of the ascitic

Variable	Subvariable	Electrophoresis pattern	
		Specific, N (%)	Non specific, N (%)
Total: N=35	All	29 (82.9)	6 (17.1)
Sex, N (%)	Male, N=26	22 (75.9)	4 (66.7)
	Female, N=9	7 (24.1)	2 (33.3)
Age (in years), N (%)	18-29	5 (17.2)	0
	30-39	2 (6.9)	0
	40-49	12 (41.4)	0
	> 50	10 (34.5)	6 (100)
Diagnosis, N=35	Liver Cirrhosis (LC)	10 (34.5)	0
	Alcoholic Liver Cirrhosis (LC)	4 (13.8)	0
	Periportal Fibrosis (PF)	2 (6.9)	0
	Nephrotic Syndrome (NPh)	3 (10.3)	0
	Hepatocarcinoma (HC)	2 (6.9)	0
	Ovarian Carcinoma (OC)	0	2 (33.3)
	Heart Failure (HF)	2 (6.9)	0
	Abd. tuberculosis	1 (3.4)	3 (50)
	Peritonitis (PT)	1 (3.4)	1 (16.7)
	Hepatitis B	4 (13.8)	0

[Table/Fig-4]: Frequency distribution of participants on the specificity of ascitic fluid electrophoresis.

fluid did not show specificity. Two cases (HF and HB) had albumin bands and showed diffuse diluted gamma bands [Table/Fig-5c].

On the other hand, among three patients (one case of NPh, one case of HB, and one case of LC), hepatitis B showed a decrease in the alpha bands and an increase in the gamma bands [Table/Fig-5d].

Among two patients with ATB, all bands showed an increase compared to normal serum [Table/Fig-5e].
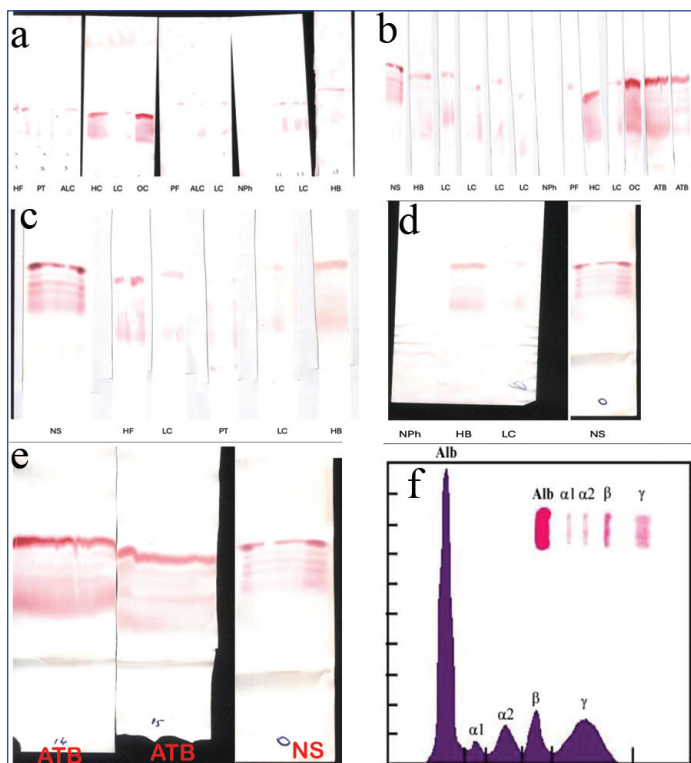
[Table/Fig-5]: (a) Ascitic fluid electrophoresis of 13 patients and their already established diagnoses , (b) 12 patients, (c) Five patients, (d) Three patients, and (e) Two patients (f) Compared to normal serum electrophoresis [12].
NS: Norm serum; HF: Heart failure; PT: Peritonitis; ALC: Alcoholic cirrhosis; HC: Hepatocarcinoma; LC: Liver cirrhosis; OC: Ovarian carcinoma; NPh: Nephrotic syndrome; HB: Hepatitis-B; PF: Periportal fibrosis; ATB: Abdominal tuberculosis

DISCUSSION

The results of the sex distribution showed that most of the patients were men. This indicates that men were more prone to alcohol consumption than women in the local community due to traditional reasons. This was consistent with a European study that shows a close link between male sex and alcoholism [17] and a Danish study that reported alcohol consumption being almost entirely confined to males in Islamic regions [18]. Half of the patients were older than 50 years, which can be taken as evidence that age was considered one of the main risk factors for ascites [19]. The study findings revealed that LC patients made up approximately one-third of the participants, which differs from the 75% reported in the literature review [20,21]. Peritonitis as a complication of ascites rather than a cause was found in 6% of cases. These findings are higher than those reported internationally [21,22]. NPh was observed only in men, which was contrary to the normal distribution ratio between sexes in another study [23] that showed in 2005 and 2006 that the incidence in boys was 60.8% and 67%, respectively. HC was seen only in men, and this may be due to the large number of patients with LC who will eventually develop HC, consistent with the literature [24]. ATB and Hepatitis B virus represent significant aetiological factors of ascites among Sudanese, which was correlated with Awasthi S et al., who reported that ATB is a main public health issue in developing countries compared to developed ones and causes considerable morbidity and mortality [25].

Ascitic fluid electrophoresis patterns among the 13 studied cases showed increased β- and diffuse γ-globulin bands. This suggests that it is possible to diagnose these cases with a specific electrophoresis pattern of ascitic fluid. Relevant data are not available in the literature for comparison.

Among patients with NPh, no bands were seen in electrophoresis. This indicates that ascites due to NPh was not suitable for diagnosis by electrophoresis. This agrees with the study that reported the failure of electrophoresis to detect serum and urine monoclonal protein [26].

HC ascitic fluid electrophoresis showed a band distribution similar to normal serum, but with an increase in the albumin band and diffuse gamma bands. This may be taken as evidence of electrophoresis specificity in detecting specific bands in this case as a diagnostic tool. A recent study raised questions regarding the utility of Alpha-Fetoprotein (AFP) in diagnosing hepatocellular carcinoma due to its complexity with multiple pathogenic mechanisms [27].

Patients with OC showed a significant increase in all ascitic fluid bands during electrophoresis, which might help identify a specific electrophoresis pattern of ascitic fluid in this condition. Relevant data for comparison are not available in the literature.

In patients with HF, there was a diluted distribution of the same bands compared to normal serum. This indicates that electrophoresis alone is not sufficient as a diagnostic tool for ascites associated with HF, possibly reflecting relatively well-preserved liver synthetic function [28].

In 12 patients diagnosed with ATB, 50% showed a specific change with an increase in the γ- band. This suggests the potential use of electrophoresis in diagnosing ATB ascites, aligning with Kashyap RS et al., who noted the diagnostic value of complex proteins in ATB ascites [29].

Among five patients, two cases with peritonitis did not exhibit specificity in ascitic fluid electrophoresis bands, leading to challenges in using this tool for diagnosis. This may be attributed to a low protein concentration as reported in other studies [30].

Conversely, in three patients with hepatitis B, there was an increase in the alpha and gamma bands. This suggests that ascitic fluid electrophoresis could be a useful tool in diagnosing these cases compared to a study that used capillary zone electrophoresis to diagnose Hepatitis C virus infection [31].

Two patients with ATB showed an increase in all bands, which may be related to the aforementioned ATB cases. This indicates that

electrophoresis alone is not sufficient as a diagnostic tool for ascites associated with ATB [28].

Ascitic fluid electrophoresis is a valuable tool for determining the diagnostic patterns of ascites due to various aetiologies. It is recommended to expand the findings of this study to include more hospitals with a larger sample size to validate the study outcomes for each specific diagnosis.

Limitation(s)

The study was conducted in only two hospitals in the state of Khartoum. A small sample size was used due to the unavailability of participants during the study period.

CONCLUSION(S)

The LC, hepatocellular carcinoma, and hepatitis B bands were specific compared to normal serum patterns, while no changes in ascitic fluid electrophoresis were observed in HF. The ascitic fluid electrophoresis was found to be useful and can be a newer tool to support the diagnosis.

Author's contribution: YO and AA conceived the original idea, YO, RE, and YK designed and collected the data, and AA analysed the data. YO, RE, and YK drafted the manuscript and AA revised it. All authors approved the final version to be published and agreed to be responsible for all aspects of the work.

REFERENCES

[1] Viji VJ. A study of serum ascites albumin gradient in the etiological diagnosis of ascites: Tirunelveli Medical College, Tirunelveli; 2010. Available from: https://azpdf.net/document/yj78mwp5-study-serum-ascites-albumin-gradient-etiological-diagnosis-ascites.html.

[2] Zaghloul AMS, Elghonimy SM, Abd Elwahab SE, Badawy AA, Ibrahim AM. Liver cirrhosis: Pathogenesis, causes, staging and management. Tob Regul Sci. 2023:4396-415.

[3] McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. Hepatology. 2021;73(Suppl 1):04-13.

[4] Matsusaki K, Aridome K, Emoto S, Kajiyama H, Takagaki N, Takahashi T, et al. Clinical practice guideline for the treatment of malignant ascites: Section summary in Clinical Practice Guideline for peritoneal dissemination (2021). Int J Clin Oncol. 2022;27(1):01-06.

[5] Goh ZNL, Teo RYL, Chung BK, Wong AC, Seak C-JJM. At the heart of the problem: Congestive cardiac failure as a cause of ascites: A narrative review. Medicine (Baltimore). 2022;101(31):e29951.

[6] Biggins SW, Angeli P, Garcia-Tsao G, Ginès P, Ling SC, Nadim MK, et al. Diagnosis, evaluation, and management of ascites, spontaneous bacterial peritonitis and hepatorenal syndrome: 2021 practice guidance by the American Association for the Study of Liver Diseases Hepatology. 2021;74(2):1014-48.

[7] Muhie OA. Causes and clinical profiles of ascites at University of Gondar Hospital, Northwest Ethiopia: Institution-based cross-sectional study. Can J Gastroenterol. Hepatol. 2019;2019:5958032.

[8] Yoshiji H. Management of cirrhotic ascites: Seven-step treatment protocol based on the Japanese evidence-based clinical practice guidelines for liver cirrhosis 2020. Hepatol Res. 2023;53(9):794-805.

[9] Oey RC, Van Buuren H, De Man RA. The diagnostic work-up in patients with ascites: Current guidelines and future prospects. Neth J Med. 2016;74(8):330-35.

[10] Dakwar GR, Zagato E, Delanghe J, Hobel S, Aigner A, Denys H, et al. Colloidal stability of nano-sized particles in the peritoneal fluid: Towards optimizing drug delivery systems for intraperitoneal therapy. Acta Biomater. 2014;10(7):2965-75.

[11] Weissinger EM, Wittke S, Kaiser T, Haller H, Bartel S, Krebs R, et al. Proteomic patterns established with capillary electrophoresis and mass spectrometry for diagnostic purposes. Kidney Int. 2004;65(6):2426-34.

[12] Luetscher JA. Electrophoretic analysis of the proteins of plasma and serous effusions. J Clin Invest. 1941;20(1):99-106.

[13] Chan PC, Chen Y, Randell EW. On the path to evidence-based reporting of serum protein electrophoresis patterns in the absence of a discernible monoclonal protein-A critical review of literature and practice suggestions. Clin Biochem. 2018;51:29-37.

[14] McPherson R, Pincus M, eds (2007) Henry's Clinical Diagnosis and Management by Laboratory Methods (Saunders Elsevier, Philadelphia), 21st Ed. E-book: Elsevier Health Sciences; 2021.

[15] Mustika S, Pratama MZ, Lesmana CRA. Diagnostic challenge in a patient presenting with ascites and hypergammaglobulinemia. Case Rep Gastroenterol. 2021;15(3):810-18.

[16] Block RJ, Durrum EL, Zweig G. A Manual of Paper Chromatography and Paper Electrophoresis. 2nd Edition, Academic Press Publishers, New York. 1958.

[17] Copenhagen Study Group for Liver Diseases. Sex, ascites and alcoholism in survival of patients with cirrhosis: Effect of Prednisone. N Engl J Med. 1974;291(6):271-73.

[18] Rehm J, Rehn N, Room R, Monteiro M, Gmel G Jernigan D, et al. The global distribution of average volume of alcohol consumption and patterns of drinking. Eur Addict Res. 2003;9(4):147-56.

[19] Guevara M, Baccaro ME, Ríos J, Martín-Llahí M, Uriz J, Ruiz del Arbol L, et al. Risk factors for hepatic encephalopathy in patients with cirrhosis and refractory ascites: Relevance of serum sodium concentration. Liver Int. 2010;30(8):1137-42.

[20] Moore KP, Aithal GP. Guidelines on the management of ascites in cirrhosis. Gut. 2006;55(suppl 6):vi01-12.

[21] Tasneem H, Shahbaz H, Sherazi BA. Causes, management and complications of ascites: A review. International Current Pharmaceutical Journal. 2015;4(3):370-77.

[22] Olivier L, Ansari N. The epidemiology of Bilharziasis. Bilharziasis: International Academy of Pathology. Special Monograph: Springer; 1967.

[23] Franke I, Aydin M, Llamas Lopez CE, Kurylowicz L, Ganschow R, Lentze M, et al. The incidence of the nephrotic syndrome in childhood in Germany. Clin Exp Nephrol. 2018;22(1):126-32.

[24] Nevola R, Tortorella G, Rosato V, Rinaldi L, Imbriani S, Perillo P, et al. Gender differences in the pathogenesis and risk factors of hepatocellular carcinoma. Biology (Basel). 2023;12(7):984.

[25] Awasthi S, Saxena M, Ahmad F, Kumar A, Dutta S. Abdominal tuberculosis: A diagnostic dilemma. J Clin Diagn Res. 2015;9(5):EC01-EC03.

[26] Kidoguchi K, Katsuya H, Ureshino H, Kizuka-Sano H, Yamaguchi K, Nagata A, et al. Concomitant nephrotic syndrome with diffuse large B-cell lymphoma: A case report. Tohoku J Exp Med. 2020;252(2):153-57.

[27] Galle PR, Foerster F, Kudo M, Chan SL, Llovet JM, Qin S, et al. Biology and significance of alpha-fetoprotein in hepatocellular carcinoma. Liver Int. 2019;39(12):2214-29.

[28] Giallourakis CC, Rosenberg PM, Friedman LS. The liver in heart failure. Clin Liver Dis. 2002;6(4):947-67.

[29] Kashyap RS, Saha SM, Nagdev KJ, Kelkar SS, Purohit HJ, Taori GM, et al. Diagnostic markers for tuberculosis ascites: A preliminary study. Biomark Insights. 2010;5:87-94.

[30] Arroyo V, Fernández J. Ascites and spontaneous bacterial peritonitis. Schiff's Diseases of the Liver. 2017:365-86.

[31] Attallah AM, Abdallah SO, El-Desouky MA, El-Far M, Omran MM, Farid K, et al. A rapid, low-cost quantitative diagnostic method for hepatitis C virus infection using capillary zone electrophoresis. Eur J Clin Microbiol Infect Dis. 2014;33(3):439-52.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Medicine, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia; Department of Biochemistry, Faculty of Medicine, University of Bahri, Khartoum, Sudan.
2. Associate Professor, Department of Pharmacology, Faculty of Pharmacy, Omdurman Islamic University, Khartoum, Sudan; Department of Pharmacology, Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia.
3. Internal Medicine Consultant, Prince Mishari bin Saud General Hospital, Baljurashi, Al-Baha, Saudi Arabia.
4. Assistant Professor, Department of Physiology, Faculty of Medicine, Al-Baha University, Al Baha, Saudi Arabia.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Aimun AE Ahmed,
Associate Professor, Department of Pharmacology, Faculty of Pharmacy,
Omdurman Islamic University, Khartoum, Sudan; Department of Pharmacology,
Faculty of Medicine, Al-Baha University, Al-Baha, Saudi Arabia.
E-mail: aimun725@hotmail.com; https://orcid.org/0000-0002-6403-132X

AUTHOR DECLARATION:
• Financial or Other Competing Interests: None
• Was Ethics Committee Approval obtained for this study?   Yes
• Was informed consent obtained from the subjects involved in the study?   Yes
• For any images presented appropriate consent has been obtained from the subjects.   Yes