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ORIGINAL RESEARCH

Spectrum of bacterial infections in ascitic fluid among established cases of chronic liver disease in a tertiary care hospital

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ABSTRACT

Background- We aimed to find out the spectrum of bacterial infections in ascitic fluid among patients of decompensated chronic liver disease in a tertiary care hospital. **Methodology**- This study was conducted as a facility based cross sectional observational study on cases of decompensated Chronic Liver disease having ascites, seeking care at People's Hospital Bhopal during the study period of 1st November 2022 to 30th April 2024. History and examination was done and findings were recorded. Diagnostic paracentesis was done and ascitic fluid subjected to examination. **Results**-Ascitic fluid biochemistry revealed low total protein levels in 47.2% cases. SAAG was less than 1.1 in 18.5% cases and more than 1.1 in 71.5% cases of decompensated Chronic Liver Disease.Gram staining revealed pus cells in 10 (8.3%) cases and Ascitic fluid culture was positive in 1.8% cases. ADA levels were raised in 1 (0.9% cases). Spontaneous bacterial peritonitis was present in 7.4% of all the sample analysed. **Conclusions**-Ascitic fluid analysis helps in evaluation of patients with Chronic Liver Disease and helps in adequate treatment and predicting the outcome of patients with decompensated chronic liver disease. We found pus cells in 10 patients out of total 108 patients on Gram stain, however, organisms were isolated in only 2 patients i.e. Enterococcus species and Pseudomonas Aeruginosa in one each.

Keywords- Decompensated liver disease, ascites, aetiology, CBNAAT, ADA.

INTRODUCTION

Chronic liver disease (CLD) is a continuous process, characterized by inflammation, destruction and regeneration of parenchyma of liver, and this repetitive process of liver injury is associated with fibrosis and ultimately cirrhosis of liver.[1] Ascites is one of the most common complications of cirrhosis, characterized by accumulation of excess fluid in the peritoneal cavity, which is pathological.[2] Approximately 5 to 7% of the compensated cirrhosis progresses to decompensated state and after a decade since diagnosis of liver cirrhosis, approximately 50% cases develop ascites. The 1 year survival rate of patients with decompensated cirrhosis is reported to be 60% whereas the 2 year survival rate is 45%.[3,4]

Ascitic cirrhosis can be complicated by subsequent bacterial infections in more than one fourth of the hospitalized patients with decompensated chronic liver disease.[5] Infection in cases with cirrhosis may further increase the risk of complications in these patients and may lead to acute on chronic liver failure.[6] The infections commonly observed in patients with decompensated chronic liver disease include spontaneous bacterial peritonitis (SBP), soft tissue infections, pneumonia, urinary tract infections (UTI), and spontaneous bacteremia.[7] The incidence of spontaneous bacterial peritonitis in cirrhotic patients have been documented to range between 7 and 30% per year, and has been associated with significant morbidity and mortality among these cases. In untreated cases, mortality can be reported in 50% cases.[8]

The higher susceptibility to bacterial infections in decompensated liver cirrhosis is attributed to several factors such as alteration of gut microflora, impaired functioning of intestinal barrier, progressive cirrhosis

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associated immune dysfunction (including immunodeficiency and systemic inflammation) etc. The cardiovascular response in decompensated liver cirrhosis cases complicated by bacteria infection is often inadequate and is responsible for rapid hemodynamic collapse.[9]

Decompensated cirrhosis complicated by infection is responsible for multiple complications such as renal failure, shock, encephalopathy etc., which can adversely affect the outcome of the patients. As these infections carry poor short as well as long term prognosis in patients with decompensated cirrhosis, it is essential to identify and treat the infection as early as possible so as to improve the outcomes in such cases. However, the typical signs and symptoms of infections may not be present and patients usually present with non-specific clinical features, preventing early diagnosis.[7,9,10] The patients with spontaneous bacterial peritonitis in decompensated cirrhosis may present with low grade fever and abdominal pain.[11,12] Thus, analysis of ascitic fluid for presence of bacterial contamination may help in early diagnosis and treatment of bacterial infection in cases with decompensated cirrhosis. The ascitic fluid must be subjected to routine microscopy and culture, gram staining, serum ascites albumin gradient (SAAG), ADA (Adenosine deaminase), CBNAAT(cartridge-based nucleic acid amplification test) etc. Early identification and treatment may improve the outcome of these patients. Only few studies have been conducted previously to detect the bacterial infection in CLD cases. Hence this study was done to make an attempt to fill up the gap in our knowledge and better understanding of such patients. With the above background, we aimed to find out the spectrum of bacterial infections in ascitic fluid among patients of chronic liver disease in a tertiary care hospital. We assessed ascitic fluid for gram staining, routine microscopy, biochemistry, culture sensitivity, SAAG (serum ascites albumin gradient), ADA (Adenosine deaminase), and CBNAAT (cartridge-based nucleic acid amplification test), for diagnosis of various bacterial infection.

MATERIALS AND METHODS

The present study was conducted as a facility based cross sectional observational study on cases of Chronic Liver disease having ascites, seeking care at People's College of Medical Science & Research Centre, and associated People's Hospital Bhopal during the study period of 18 months i.e. from 1st November 2022 to 30th April 2024 in the Medicine department.All cases of chronic liver disease with ascites admitted during the study period willing to participate in the study were included in the study whereas patients with compensated liver disease, malignancy (hepatocellular carcinoma), bleeding disorder, those who received antibiotics within last 7 days and patients with ascites not due to chronic liver disease (systemic illness like Nephrotic syndrome, congestive cardiac failure, Primary hypothyroidism etc.) were excluded from the study.

After obtaining ethical clearance from Institute's ethical committee, all the patients with CLD with ascites satisfying inclusion and exclusion criteria were enrolled in our study. Detailed history regarding sociodemographic variables, presenting complaints, addiction, drug history, past medical/ surgical history, and comorbidities if any was obtained and documented. All the patients were then subjected to detailed general physical and systemic examination.

Patients were then subjected to routine blood investigations (including LFT) along with USG whole abdomen

All the patients were then subjected to diagnostic paracentesis. Under all aseptic precautions, using a 16-18 gauge needle (Spinal needle for obese patients), 20 cc or 50 cc syringe, sample of ascitic fluid was collected in respective containers. Diagnostic aspiration of fluid (ascitic fluid) was done and subjected to routine microscopy, Gram staining, Culture, Biochemical analysis, SAAG, ADA, CBNAAT.

STATISTICAL ANALYSIS

Data was compiled using MS Excel and analysis was done using IBM SPSS software version 20. Categorical and continuous data were expressed as frequency (%) and mean (±standard deviation). Association of pus cells with various factors was done using chi square test. P value of less than 0.05 was considered statistically significant.

RESULTS

The present study was conducted on a total 108 patients with Decompensated chronic liver disease with mean age of 48.17±14.726 years. About 72.2% of cases were males.History of alcohol addiction was present in more than half of the cases (53.7%), of them history of addiction of alcohol with other substances was present in 27.8%. Apart from this, history of addiction to other agents was present in 24.1% cases. All the patients with CLD presented with abdominal distension (100%), and 53.7% cases presented with other complaints such as shortness of breath (11.1%), bilateral pedal edema (11.1%), generalized weakness (7.4%), yellowish discoloration of eyes (6.5%) and blood in vomitus (3.7%). About 2.8% cases each presented with history of black tarry school and vomiting. Small proportion of cases (1 case each) presented with history of cough, left side hemiparesis, esophageal varices, history of COPD, hypertension, inability to talk, type 2 diabetes and weight loss. 49.07% cases were of Alcoholic Liver Disease. About 35.18% cases were of other (undetermined [genetic causes (Alpha-1 antitrypsin deficiency, Wilson disease, Hemochromatosis), Autoimmune hepatitis, Primary biliary cirrhosis (PBC), Primary Sclerosing Cholangitis (PSC)] causes (Table 1).

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Table 1- Distribution of cases according to baseline variables

Baseline variables		Frequency (n=108)	Percentage
Age (years)	Mean±SD	48.17±14.726	
Sex	Male	78	72.2
	Female	30	27.8
Addiction	Alcohol	28	25.9
	Alcohol with tobacco	13	12
	Tobacco chewer	17	15.7
	Alcohol, tobacco and other	1	0.9
	Tobacco and other	1	0.9
	Alcohol with other	16	14.8
	Others	8	7.4
	None	24	22.2
Complaints	Abdominal distension	108	100.0
	Abdominal Pain	70	64.8
	Fever	13	12.0
	Others	58	53.7
Aetiology	Alcoholic Liver Disease	53	49.07
	NAFLD/NASH	5	4.6
	Chronic Viral Hepatitis	12	11.11
	Others	38	35.18

Table 2- Distribution according to findings of Ascitic fluid in patients with CLD

Ascitic fluid			Frequency (n=108)	Percentage
SAAG	<1.1		20	18.5
	>1.1		88	81.5
Routine Microscopy	Total cells	<250	100	92.6
		>250	8	7.4
Pus cells on Gram stain		Absent	98	90.7
		Present	10	9.3
Culture		Enterococcus species	1	0.9
		Pseudomonas Aeruginosa	1	0.9
		Sterile	106	98.2
ADA		<40	107	99.1
		>40	1	0.9
CBNAAT		Negative	45	41.6
		Positive	0	0.0
		Not done	63	58.33

SAAG was less than 1.1 in 18.5% cases of CLD.Mean total cell count in cases with CLD was 208.4±601.922. Gram staining revealed pus cells in 10 (8.3%) cases and Ascitic fluid culture was positive in 1.8% cases i.e. 0.9% cases each ascitic fluid culture revealed growth of Enterococcus species and Pseudomonas Aeruginosa. ADA levels were raised in 1 (0.9% cases) and mean ADA levels in CLD cases was 4.90±5.41. CBNAAT (Cartridge based nucleic acid amplification test) was done in 45 cases and it was negative in all the cases (Table 2).

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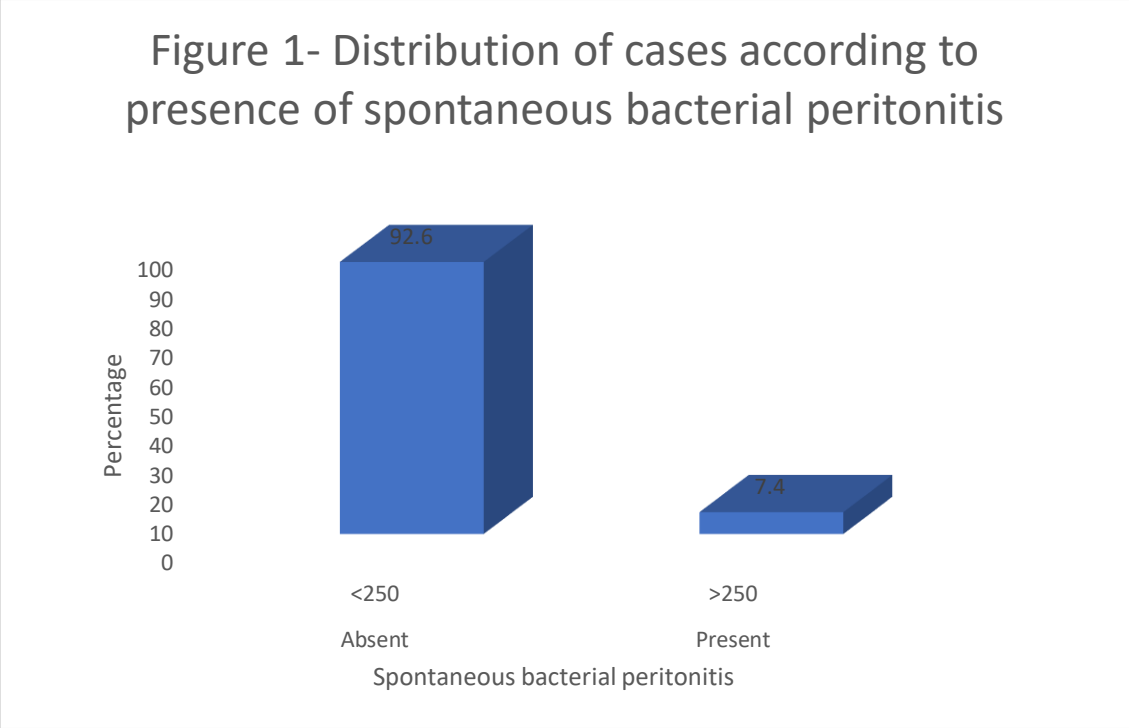

Spontaneous bacterial peritonitis was present in 7.4% of all the sample analysed (Figure 1).

DISCUSSIONS

Ascitic fluid analysis may aid in diagnosing various infections in patients with decompensated cirrhosis. Bacterial infections have been reported to be common in patients with CLD and their presence is associated with poor prognosis and short term mortality.[12-14] We performed ascitic fluid analysis in patients with CLD to determine the bacterial infections in our study group. Ascitic fluid was subjected to biochemical analysis, SAAG, culture, ADA, CBNAAT, routine microscopy and gram staining. Ascitic fluid examination revealed protein levels of less than 2.5 g/dl in 47.2% cases with mean protein levels of 2.40±1.4 g/dl whereas mean albumin levels were 0.79±0.78g/dl. Mean glucose levels in ascitic fluid analysis in patients with decompensated CLD were 115.62±30.1 mg/dl. However, in a study of Maskey et al, mean proteins and sugar in ascitic fluid analysis were 1.408±2.687 mg/dl and 127.24±66.5 mg/dl respectively.[15] Vaz et al presented a case of TB peritonitis, in which the authors found proteins to be elevated with slightly elevated SAAG.[16]

In present study, we assessed SAAG and found serum albumin to be low in 85.2% cases and mean serum and ascitic albumin levels were 2.52±0.742 and 0.75±0.712 g/dl respectively. We found SAAG to be raised in 81.5% cases in our study population, suggestive of portal hypertension. However, because its accuracy only varies from 87.5 to 97%, SAAG may be falsely low in some situations. Unexpectedly low SAAG ascites can be caused by significantly low levels of serum albumin.[17] According to Subhaniet al, when used to diagnose portal hypertension as the etiology of ascites, SAAG ≥11 g/L showed a sensitivity of 85.5% and a specificity of 60.6%.[18] Vaz et al observed slightly elevated SAAG in patient with TBP along with elevated protein levels.[16]

In present study, ascitic fluid was also subjected to routine microscopy and we observed cell counts to be raised (>250) in 7.4% cases. Mean cell count in patients with CLD was 208.4±601.922 cells. Overall, mean PMNs and mean Lymphocytes levels in ascitic fluid were 34.56±33.73% and 59.76±33.80% respectively. Mean TLC, neutrophils and lymphocyte counts in patients with CLD in a study of Maskey et al were 9180.22±2518.62, 7000.65±2325.14 and 219.46±417.18 mm^3 respectively.[15] Mean WBC count in patients with cirrhosis in a study of Samonakis et al was 1413.5±2232 cells, which was much higher as compared to present study, reflecting higher severity of infections.[19] .

Gram stain revealed pus cells in 9.3% cases and majority of cases had 8 to 10 pus cells (3.7%). Chinock et al documented gram stain to be positive in 3.9% cases and the sensitivity of gram stain was documented to be 10% whereas specificity was 97.5% for diagnosis of SBP.[20]

Ascitic fluid culture revealed growth of micro-organisms suggesting underlying infections in 1.8% cases (Enterococcus species and Pseudomonas Aeruginosa in 1 case each) of decompensated liver cirrhosis. Though we found pus cells in 9.3% but bacteria could be cultured in only 1.8%. As few patients were unable to recall their history of antibiotic use thus may have taken antibiotics before we obtained ascitic fluid sample for culture. The yield of ascitic fluid culture is high when sample is obtained prior to prescription of antibiotics.[21]Doddamani et al

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observed Escherichia.coli as the most common cultured organism in patients with SBP (50%), followed by Klebsiella (37.5%) and Pseudomonas aeruginosa (12.5%).[13]Bibi et al reported ascitic fluid culture positivity rate of 50% and the most common organism was E. coli (65%) followed by Enterococcus species (15%).[12]Purohit et al found culture positivity rate of 43.6% in their study and E. coli was the most common microorganism observed in 54.9% cases.[8]

In our study, incidence of spontaneous bacterial peritonitis in patients with decompensated liver cirrhosis was 7.4%. The findings of present study were supported by the findings of Doddamani et al, where the authors found SBP in 9.8% cirrhotic patients.[13]Naqvi et al documented ascitic fluid infections in 44.89% cases of CLD.[14] Bibi et al reported SBP in much higher proportions of cases with decompensated cirrhosis (24.2%) as compared to present study.[12]

Ascitic fluid was also subjected to ADA estimation and CBNAAT to observe presence of TB peritonitis in our study group. We found ADA levels to be raised in 1 case with mean levels of 4.90±5.41 and CBNAAT was negative in all the cases who were subjected to CBNAAT. Vaz et al presented a case of TB peritonitis in which the authors documented ADA levels to be more than 40, however, the ZN stain and culture on LJ medium was negative.[16] Kumabe et al found Fifteen patients (median, 87.2 IU/L; range, 44.0–176.1 IU/L) exhibited elevated ascitic ADA levels (≥40 IU/L); eight of them had tuberculous peritonitis. For the diagnosis of peritoneal TB, increased ascitic fluid ADA levels (≥40 IU/L) had 100% sensitivity and NPV whereas it has 96% specificity and 53.3% PPV.[22] Soni et al observed CBNAAT to be positive in only 8.3% cases of TBP and the diagnostic accuracy of ADA was 61.6% and that of CBNAAT was 43.2% for diagnosis of extrapulmonary TB.[23]

CONCLUSIONS

Based upon the findings of presenting study, it could be concluded that ascitic fluid analysis helps in evaluation of patients with CLD and help in adequate treatment and predicting the outcome of patients with decompensated liver disease. We found pus cells in 10 patients out of total 108 patients on gram stain, however, organisms were isolated in only 2 patients i.e. Enterococcus species and Pseudomonas Aeruginosa.

LIMITATION OF THE STUDY

The study had certain limitations, first, the study was conducted as a facility based cross sectional study in single centre, and thus the findings cannot be generalized. A large multicentric study with higher sample size is required to validate the findings of our study.

RECOMMENDATION

In patients of established decompensated chronic liver disease with ascites superadded infections were seen in 9.3% cases in ascitic fluid. These patients were mostly males, addicted to alcohol, tobacco, were malnourished, and were having low BMI. Most of these patients had presenting complaint of abdominal pain.Hence, it is recommended that patients with above characteristics are more prone to develop infections and hence we should keep a strict vigil to look for superadded infections in this subset of patients.

REFERENCES

1. Sharma A, Nagalli S. Chronic Liver Disease. [Updated 2022 Jul 4]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554597/
2. Chiejina M, Kudaravalli P, Samant H. Ascites. [Updated 2022 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470482/
3. Ginés P, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. Hepatology. 1987 Jan;7(1):122-8.
4. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. Journal of hepatology. 2006 Jan 1;44(1):217-31.
5. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, Burroughs AK. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology. 2010 Oct 1;139(4):1246-56.
6. Trebicka J, Fernandez J, Papp M, Caraceni P, Laleman W, Gambino C, Giovo I, Uschner FE, Jimenez C, Mookerjee R, Gustot T. The PREDICT study uncovers three clinical courses of acutely decompensated cirrhosis that have distinct pathophysiology. Journal of hepatology. 2020 Oct 1;73(4):842-54.
7. Fasolato S, Angeli P, Dallagnese L, Maresio G, Zola E, Mazza E, Salinas F, Dona S, Fagiuoli S, Sticca A, Zanus G. Renal failure and bacterial infections in patients with cirrhosis: epidemiology and clinical features. Hepatology. 2007 Jan;45(1):223-9.
8. Purohit PH, Malek SS, Desai KJ, Mihir S. A study of bacteriological profile of ascitic fluid in suspected clinical cases of spontaneous bacterial peritonitis at a tertiary care hospital in India. International Journal of Medical Science and Public Health. 2015;4(4):496-501.
9. Albillos A, Lario M, Álvarez-Mon M. Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance. Journal of hepatology. 2014 Dec 1;61(6):1385-96.
10. Jalan R, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, Stadlbauer V, Gustot T, Bernardi M, Canton R, Albillos A. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. Journal of hepatology. 2014 Jun 1;60(6):1310-24.
11. Bhat G, Vandana KE, Bhatia S, Suvarna D, Pai CG. Spontaneous ascitic fluid infection in liver cirrhosis:

DOI: 10.69605/ijlbpr_14.2.2025.177

bacteriological profile and response to antibiotic therapy. Indian Journal of Gastroenterology. 2013 Sep;32:297-301.
12. Bibi S, Ahmed W, Arif A, Khan F, Alam SE. Clinical, laboratory and bacterial profile of spontaneous bacterial peritonitis in chronic liver disease patients. J Coll Physicians Surg Pak. 2015 Feb 1;25(2):95-.
13. Doddamani GB, Pujar S, Kora SA. Spontaneous Bacterial Peritonitis in Ascites: A prospective study in a tertiary care hospital. J Clin Diagn Res. 2010;4:2737-41.
14. Naqvi IH, Mahmood K, Talib A, Ubaid M, Mahmood A. Infections in Cirrhotics: Types, Microbiological Spectrum and Risk Factors—5-Year Cohort Study. Open Journal of Gastroenterology. 2014 Mar 6;2014.
15. Maskey R, Karki P, Ahmed SV, Manandhar DN. Clinical profile of patients with cirrhosis of liver in a tertiary care hospital, Dharan, Nepal. Nepal Med Coll J. 2011 Jun 1;13(2):115-8.
16. Vaz AM, Peixe B, Ornelas R, Guerreiro H. Peritoneal tuberculosis as a cause of ascites in a patient with cirrhosis. BMJ Case Rep. 2017 Jul 14;2017:bcr2017220500.
17. Trongtorsak A, Kittipibul V, Antala D, Meng Q, Puwanant S. Heart Failure-Related Ascites With Low Serum-Ascites Albumin Gradient: Diagnostic Clues From Triphasic Abdominal Computed Tomography. Cureus. 2022 Jan;14(1).
18. Subhani M, Sheth A, Palaniyappan N, Sugathan P, Wilkes EA, Aithal GP. Diagnostic accuracy of serum ascites albumin gradient (SAAG) in a contemporary unselected medical cohort. Journal of International Medical Research. 2022 Nov;50(11):03000605221140310.
19. Samonakis DN, Gatselis N, Bellou A, Sifaki-Pistolla D, Mela M, Demetriou G, Thalassinos E, Rigopoulou EI, Kevrekidou P, Tziortziotis I, Azariadi K. Spontaneous bacterial peritonitis: a prospective Greek multicenter study of its epidemiology, microbiology, and outcomes. Annals of Gastroenterology. 2022 Jan;35(1):80.
20. Chinnock B, Fox C, Hendey GW. Gram's stain of peritoneal fluid is rarely helpful in the evaluation of the ascites patient. Annals of emergency medicine. 2009 Jul 1;54(1):78-82.
21. Rojo M. Collect before you treat: obtaining cultures before antibiotic treatment'. Drugs & Therapy Bulletin. 2006;20(10):1-3.
22. Kumabe A, Hatakeyama S, Kanda N, Yamamoto Y, Matsumura M. Utility of ascitic fluid adenosine deaminase levels in the diagnosis of tuberculous peritonitis in general medical practice. Canadian Journal of Infectious Diseases and Medical Microbiology. 2020;2020(1):5792937.
23. SonI AK, PURASKAR P, ShrIKhAnde A, SonI S. Efficacy of CBNAAT versus Adenosine Deaminase in Fluids in Extrapulmonary Tuberculosis. Journal of Clinical & Diagnostic Research. 2022 Mar 1;16(3).