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Original Research Article

Microbial Profile of Liver Abscess in Patients Attending Surgery Department at Tertiary Care Centre

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Abstract:

Background: Worldwide, liver abscess is known disease commonly caused by parasitic origin (amoebic) in developing countries while pyogenic in developed countries. In this study, we wanted to evaluate the microbial spectrum of liver abscess, its etiological causes, the drug susceptibility pattern of the isolates from the liver abscess and the outcome of the disease.

Materials and Methods: This was a prospective cross-sectional study conducted in the Department of Microbiology among 90 patients diagnosed with a liver abscess in the Department of Surgery of a tertiary care hospital from January 2019 to June 2020.

Results: Amoebic liver abscess was the predominant (71%) etiological cause of the liver abscess. E. coli was the most common (11%) causative organism of pyogenic liver abscess. Middle-aged males were common and the majority of the patients belonged to poor socioeconomic status and were chronic alcoholics. The main symptoms were fever (89%) and abdominal pain (62%) with hepatomegaly (58%) as a common sign. Complications like rupture of an abscess (four cases), sepsis (four cases), chronic liver disease (two cases) and liver failure (two cases) were observed in the study. In this study, the management modality included starting patients on an initial empirical antimicrobial regime. Along with this minimally invasive abscess drainage was carried out in the majority of cases either by fine-needle aspiration (42%) or pigtail drainage (27%). Few complicated cases were required to be managed with open surgery (11%).

Conclusion: In India, amoebic liver abscess remains the more common type of liver abscess as compared to pyogenic liver abscess. E.coli is the most common organism isolated among the pyogenic abscesses. Solitary right lobe abscess was the most common pattern in our study. Early and appropriate antimicrobial treatment and drainage of the abscess have resulted in an improved outcome of the disease.

Keywords: Microbiological Profile, Amoebic Liver Abscess, Antibiotic Susceptibility Pattern, Liver Abscess, Outcome.

Introduction

A liver abscess (LA) is defined as a collection of purulent material in the liver parenchyma, which can be due to bacterial, parasitic, fungal, or mixed infection including anaerobic and tubercular infection. [1] It is a common condition across the globe. Out of the total incidence of LA, approximately two-thirds of cases in developing countries are of amoebic aetiology and three-fourths of cases in developed countries are pyogenic. [1] Liver abscess (LA) is defined as collection of purulent material in liver parenchyma, which can be due to bacterial, parasitic, fungal, or mixed infection including anaerobic and tubercular infection. [1] It is a common condition across the globe. Out of total incidence of LA, approximately two-thirds of cases in developing countries are of amoebic etiology and three-fourths of cases in developed countries are pyogenic. [1] In tropical countries, parasitic causes of Liver abscesses predominates, most frequently due to Entamoeba histolytica (E. histolytica) resulting in Amoebic Liver Abscess (ALA). [2,3] Amebiasis is endemic in the tropical region due to poor sanitation, unsafe drinking water and overcrowding.(1) It often

presents with nonspecific symptoms and diagnosis is often delayed. [4] Pyogenic liver abscess (PLA) is a highly lethal condition. [5] It occurs secondary to biliary or intestinal tract infections, hematogenous seeding or extension of contiguous infection, and carries a mortality rate of 20–60% even with appropriate medical–surgical management. [5] Predominantly isolated organisms include Escherichia coli, Klebsiella pneumoniae, Streptococcus sp. or Staphylococcus sp. and anaerobic organisms. [6–8] Over the past two decades, new investigation methods, such as ultrasound, computed tomographic scanning, direct cholangiography, guided aspiration, and percutaneous drainage, have helped early diagnosis and treatment of these patients. [8] Liver abscess may result in severe complications and even mortality.

Mortality fluctuates between 8% and 31%. [9,10] It is now decreasing due to early diagnosis, the use of more effective antibiotics, and percutaneous drainage (percutaneous puncture or puncture with a fine needle) with ultrasound or CT guidance. [11] Liver abscess due to fungal infections are most commonly caused by Candida spp., often occur in patients with Hematologic malignancies treated with chemotherapy and Aspergillus spp. is also known to cause liver abscess in some patients. [12,13] Tubercular Liver Abscess (TLA) are uncommon and are normally associated with primary foci of infection in the pulmonary system or gastrointestinal tract. [14] The incidence of TLA has increased in recent past due to increased incidence of predisposing factors like alcoholism, immunodeficiency, irrational usage of antibiotics, and emergence of drug resistant bacilli. [1] The, knowledge of etiology and timely treatment of underlying cause plays an important role in improved outcome of the disease.

Hence, the present study is conducted to determine the varied etiology of liver abscess and to study the drug susceptibility pattern of the bacterial isolates from liver abscess.

Material and Methods

This was a prospective cross-sectional study conducted in the Department of Microbiology among patients diagnosed with a liver abscess in the Department of Surgery of a tertiary care hospital from January 2019 to June 2020. Institutional ethics committee permission was obtained before the commencement of the study. The patients were informed about the study and informed consent was taken. A total of 90 consecutive patients with a liver abscess were studied from January 2019 to June 2020 (18 months).

Study Procedure

Pus specimen was collected by USG guided aspiration. Some quantity of the sample was immediately inoculated in laboratory prepared thioglycolate medium while some were sent for routine microbiological evaluation. This pair of specimens was sent to microbiology lab within 20 mins of collection time. Once the specimen was received in a microbiology lab, part of it was used for direct microscopy by wet mount preparation for examination of trophozoite of E. histolytica (within half-hour of specimen collection). Other part of the specimen was used for evaluating microbes by using different staining and culture methods.

Gram staining preparations were performed to study the cellular and bacterial morphology. Samples were cultured both aerobically and anaerobically as per standard protocol. For aerobic culture, 5 % Sheep blood agar (Himedia, Mumbai) and MacConkey's agar were used and incubated at 37°C for 24 h. Antimicrobial susceptibility testing was carried out on Mueller-Hinton agar by Kirby Bauer disc diffusion method and interpreted as per CLSI guidelines. [15] For anaerobic culture, laboratory prepared Thioglycolate broth was used. GasPak anaerobic system was used to create an oxygen-free environment for the growth of the anaerobic organism. [16] Specimens were also cultured on Sabouraud dextrose agar (SDA) as per standard protocol to demonstrate any fungal aetiology.

Ziehl- Neelsen staining was performed to demonstrate the presence of any acid-fast bacilli. For a demonstration of Entamoeba histolytica aetiology, along with wet mount ELISA was performed to demonstrate E. histolytica antigen from the aspirate specimen. [17] All the pus specimens were tested by Qualitative Human Entamoeba histolytica Antigen (EHAg) ELISA Kit (by MyBioSource), as per the procedure mentioned in the kit literature. This is an ELISA based antigen detection kit used only for research purposes. The reports of this test were not provided to the patients.

Results

Out of the total 90 liver abscess cases included in this study, 80 (89 %) were males and 10 (11 %) were females, with a male-female ratio of 8:1. In this study, the age distribution of liver abscess cases ranged from 20 years to 73 years, with maximum cases in the age group of 31 yrs-40 yrs (42 %), followed by > 50 yrs (24%) and 41 yrs-50 yrs (20 %).

Of the 90 cases, 12 cases (13.3%) presented with complications such as ruptured liver abscess (n=4), liver abscess with sepsis (n=4), liver abscess with liver failure (n=2) and chronic liver disease (n=2).

Majority of the patients presented with fever (89 %), abdominal pain (62 %), hepatomegaly (58 %) and abdominal tenderness (53 %). Comorbidities were associated in 66 of 90 patients (73.3%) with predominant being chronic alcoholism (48 cases), diabetes mellitus (4 cases), combined diabetes mellitus and chronic alcoholism (10 cases) and HIV (four cases). Characteristic anchovy sauce appearance was seen in 78% cases (n=70) followed by purulent appearance in 22% cases(n=20). 78% of cases (n=70) had a singular loculated abscess and 22% (n=20) presented with multiple abscesses. Other deranged laboratory findings were raised leucocytes (80%), raised alkaline phosphatase (74%), raised ESR (70%), low haemoglobin levels (67%), raised SGOT (60%), raised SGPT (49%) and lastly, raised bilirubin (33%).

USG findings showed variable size of liver abscesses. 38 % cases had size of 2 cm-4 cm, followed by 31 % cases with size of < 2 cm and > 4 cm respectively. The management of liver abscess varied with 42% were managed by USG guided fine needle aspiration (42 %), followed by pigtail drainage (27 %) and open surgery (11%). Also, 20 % of cases were managed conservatively.

Microbiological Investigations

Serological tests:

The serological investigation of corresponding cases demonstrated ELISA positive (for E. histolytica antigen) in 62 cases among the single loculated abscess cases and 2 cases among the multiple abscess cases.

Microscopy

All aspirated pus specimens (n=90) did not demonstrated motile trophozoites of E. histolytica on wet mount and did not showed any acid fast bacilli on Ziehl-Neelsen (ZN) staining while Gram's staining revealed pus cells and no organism in 79 % cases, pus cells and Gram-negative bacilli 15 % cases, pus cells and gram-positive cocci in pairs in 4 % cases and 2 % cases with pus cells and budding yeast cells.

Culture findings:

Culture of the abscess demonstrated growth in 19 cases out of 90 patients (21.1%), of which 10 cases were Escherichia coli, two cases each of Proteus mirabilis, Enterococcus faecalis and Staphylococcus aureus and one case of Burkholderia pseudomallei. Two cases demonstrated fungal growth of Candida tropicalis. No anaerobic organisms were isolated in the study.

Antibiotic susceptibility pattern findings:

Out of 10 cases of E. coli, six cases were susceptible to ampicillin-sulbactam and aminoglycosides like amikacin, netilmicin, and gentamicin and only two cases were susceptible to other antimicrobials like cefepime, ceftriaxone, Cotrimoxazole, piperacillin-tazobactam, imipenem, meropenem, ciprofloxacin and levofloxacin respectively. Two cases of Proteus mirabilis were susceptible to ampicillin-sulbactam, amikacin, netilmicin, gentamicin, cefepime, cotrimoxazole, piperacillin-tazobactam, imipenem and meropenem each. One case of Burkholderia pseudomallei isolated was susceptible to ceftazidime, cotrimoxazole and meropenem. Two cases demonstrated growth of Staphylococcus aureus, which was susceptible to amoxiclav, methicillin, netilmicin, gentamicin, cotrimoxazole, tetracycline, teicoplanin, linezolid, erythromycin and clindamycin each. Enterococcus faecalis was also isolated from two cases, susceptible to penicillin, ampicillin, ciprofloxacin, teicoplanin, linezolid, vancomycin and nitrofurantoin each.

In this study, the blood culture of 38 patients was received. A majority (34 cases) of them were negative for culture findings. Staphylococcus aureus was isolated from two cases and Candida tropicalis from two cases. Corresponding patient's liver abscess aspirate culture demonstrated the same isolates respectively.

Antigen detection by ELISA:

ELISA for detection of E. histolytica antigen was performed on aspirates of all liver abscess cases (n=90), 64 cases (71%) were ELISA positive.

Comorbidities and outcome:

Out of 90 cases, 80 patients recovered and were discharged. 10 patients died, of which six cases were complicated and also had associated comorbidities. A total of 66 cases were associated with comorbidities either singularly or multiple comorbidities together, among which 10 cases were deceased. (p=0.073) Out of 90, only 12 cases were complicated, among these six patients were deceased. (P < 0.001)

Discussion

The predominant causative organism of liver abscess in the present study was amoebic (71%) followed by pyogenic (19%). In 8% of cases, no etiological organism could be demonstrated, which could be due to patients receiving prior antibiotic treatment before reaching this tertiary care centre. An amoebic liver abscess (ALA) is a predominant pathogen as also reported in studies by Shah Naveed et al. and Soumik Ghosh et al, in India. [1,2] In the pyogenic liver abscess, E.coli (11%) was the most common microorganism detected. This finding was similar to studies by Naveed et al and Wasif Mohammad Ali et al. [2,18]

Based on the findings of Ziehl–Neelsen staining of pus specimen, no case of M. tuberculosis was

detected. This result could be biased as no other culture/molecular diagnostic test was carried out and no histopathological studies on liver abscess tissue were performed. Other studies from the tropical region by Prahlad Karki et al. [19] and Shah Naveed et al. [2] demonstrated 5.5% and 3.3% tubercular liver abscess respectively.

One case of melioidosis was demonstrated in this study. The patient was a farmer from a rural area. Melioidosis is an important cause of liver abscess in Southeast Asia. [20] The infection is caused by Burkholderia pseudomallei, a saprophytic gram-negative bacillus found in the environment. Similar findings were reported in studies by Yu-Lin Lee et al. [21] from Taiwan and by RR Maude et al. [22] from Thailand. No growth of anaerobic organisms could be demonstrated in the present study. This may be due to patients receiving prior antibiotic treatment before reaching this tertiary care centre. A study by Sayek et al. also reported that anaerobic bacteria are seldom cultured. [23]

The present study had found two cases of fungal liver abscess with the causative organism as Candida tropicalis in patients with a history of uncontrolled diabetes and positive blood culture (candidemia). Candidiasis of the liver is an unusual infection that occurs almost exclusively in individuals with underlying defects in host defense mechanisms such as in cases of diabetes mellitus, leukaemia and chronic granulomatous disease. [24]

In this study, it was observed that liver abscess was more common in males as compared to females (ratio of 8:1). Most of the cases fall in the age group of 31yrs-40yrs, i.e. 42 % which was in accordance with Indian studies by Sharma et al. [25] and Mukhopadhyay et al. [26] The majority of the patients were of rural and poor socioeconomic status, and most of the male patients were chronic alcoholics (64%).

The age predisposition and gender differences may be a result of high alcohol intake by men which predisposes to ALA. Alcohol suppresses the function of Kupffer cells (Specialized macrophage) in the liver which has an important role in clearing amoeba. [26] Moreover, invasive amoebiasis appears to be dependent on the availability of free iron. A high content of iron in the diet, often obtained from the country liquor in habitual drinkers predisposes to invasive amoebiasis, as does a diet rich in carbohydrates.

Elderly individuals with underlying diseases and patients with compromised immunity due to malnutrition or corticosteroid therapy are also prone to invasion by amoeba. [1] Moreover, Reddy and Thangavelu proposed that the female menstrual cycle prevents hepatic congestion and thus makes the organ less susceptible to abscess formation. [27] Fever (89 %) and abdominal pain (62 %) were the most common symptoms seen while tender hepatomegaly (58 %) was the most common sign noted. This was in accordance with studies by Wasif Mohammad Ali et al. (18) and Prahlad Karki et al. [19]

Diarrhoea was seen in 16 % of cases, all were ALA. Satyarth Chaudhary et al. also reported diarrhoea in 7 % of cases of ALA. [28] Chronic Alcoholism (64 %) was the most common comorbidity associated with 58 cases of liver abscess followed by diabetes mellitus (13%). There were four patients who tested positive for HIV. Similar findings were seen in a study conducted in Central India by Chaudhary et al. [28] Blood investigations showed anaemia (67 % cases) and leukocytosis (80 % cases). Liver enzymes, especially serum alkaline phosphatase were raised (74 % cases). Deranged SGOT values were seen in 56 % of cases, whereas SGPT values were normal in 51 % of cases. Bilirubin was normal in 67 % of cases. ESR was raised in 70 % of cases. Soumik Ghosh et al. reported similar findings. [1]

In this study, the blood culture of 38 patients was received. A majority (34 cases) of them were negative for cultural findings. Staphylococcus aureus was isolated from two cases and Candida tropicalis from two cases. In these patients, liver abscess aspirate also demonstrated identical culture findings. These patients had developed septicaemia and candidemia respectively, rare complications seen in pyogenic liver abscesses and fungal liver abscesses. This was in accordance with findings reported by Samuel Igbinedion et al. [29] Another study by Pamela A. Lipsett et al. [30] reported fungemia as a fatal complication in cases of fungal liver abscess.

In the present study, the majority of cases presented clinical features which did not allow a definitive diagnosis of the type of abscess. Hence, the patients were started with initial conservative treatment with the antimicrobial regime that included a combination of metronidazole with ampicillin-sulbactam and gentamicin for coverage of Entamoeba histolytica other aerobic and anaerobic organisms.

When the culture results and serological results were obtained, the antibiotics were altered, as appropriate. Consistent with the latest management strategy of minimally invasive drainage techniques, USG guided needle aspiration was done in most patients (42%) and Pigtail drainage in some patients (27%).

Appropriate antimicrobials were added according to the etiological outcome. However, 11% (n=10) of the patients had to undergo surgical intervention as 4 cases (4%) got complicated with rupture. The management approach adopted by Soumik Ghosh et al. in their study mainly included fine needle

aspiration (79 %) followed by pigtail drainage (17 %) whereas only 4 % of patients required open surgical drainage. [1] Once the diagnosis of the amoebic liver abscess was established, empirical treatment with metronidazole was followed by another agent paromomycin, to treat the luminal carrier state that occured in 40 to 60 % of patients. Irusen EM et al. [31] applied a similar treatment approach in their study.

It was observed that the most common isolated organism among pyogenic liver abscesses in the present study, was E. coli (10 cases), which are part of gut flora and are believed to seed into liver parenchyma via the portal circulation. [20] E. coli demonstrated susceptibility to ampicillin-sulbactam and aminoglycosides such as amikacin, netilmicin and gentamicin in 60 % of cases. Its susceptibility to cefepime, ceftriaxone, cotrimoxazole, piperacillin tazobactam, imipenem, meropenem, ciprofloxacin and levofloxacin was only 20 %. One case of Burkholderia pseudomallei isolated was susceptible to ceftazidime, cotrimoxazole and meropenem. Staphylococcus aureus isolated from two cases was susceptible to amoxiclav, methicillin, netilmicin, gentamicin, cotrimoxazole, tetracycline, teicoplanin, linezolid, erythromycin and clindamycin each. Enterococcus faecalis was also isolated from two cases, susceptible to penicillin, ampicillin, ciprofloxacin, teicoplanin, linezolid, vancomycin and nitrofurantoin respectively. According to this drug susceptibility pattern, antibiotic treatment was modified in cases of pyogenic liver abscess.

In patients with fungal liver abscesses, surgical drainage was carried out followed by the direct intralesional administration of liposomal amphotericin B. Cinzia Auriti et al. managed the cases of fungal liver abscess in their study by this method. [32] With the above management modalities, the overall mortality rate in the present study was 11%, (10 patients out of 90 were deceased). Here out of 90 cases, 66 cases were associated with comorbidities like chronic alcoholism, diabetes mellitus and HIV, either singularly or multiple comorbidities together, among which 10 patients expired. ($P = 0.073$) Thus the mortality rate was not significantly related to the presence of comorbidities. Prahlad Karki et al. reported an overall mortality rate of 5.5 % in their study. [19] Whereas, out of 90 cases in the present study, only 12 cases were complicated with rupture of an abscess (four cases), sepsis (four cases), chronic liver disease (two cases) and liver failure (two cases). Among these complicated patients, six patients expired. ($P < 0.001$) Thus above findings indicate that the mortality was higher in patients with associated complications.(19) The mortality rate was 12 % in the study conducted by F. Reyna Sepulveda et al. [33] Mortality rates in the year 1991- 2001, before the new treatments were 50-77 %. [34] Nowadays, with modified treatment approaches like percutaneous needle aspiration and pigtail drainage of the abscess, mortality is reduced to 1-3 % [35] in amoebic liver abscess and to 10 % in pyogenic liver abscess. [36]

Conclusion

In India, amoebic liver abscess remains as the more common type of liver abscess as compared to pyogenic liver abscess. E.coli was the most common organism isolated among the pyogenic abscesses. Solitary right lobe abscess was the most common pattern in our study. Early and appropriate antimicrobial treatment and drainage of the abscess have resulted in an improved outcome of the disease. Mortality was high in patients with associated complications. Overall mortality was low probably due to the use of minimally invasive drainage techniques and aetiology-specific antimicrobials in all patients.

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