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A Cross Sectional Study to Assess Clinical Profile of Patients with Liver Abscess in Tertiary Care Centre at Central Rajasthan

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Abstract

Introduction: A liver abscess is infective disease of liver parenchyma having collection of purulent material that can develop as a consequence of injury to the hepatic tissue or via portal vein from any source of infection within abdominal cavity. Mostly liver abscess are either due to amoebic infection or pyogenic bacterial infection but in some cases, etiologies like fungal, tubercular or sometimes mixed infections is also seen.

Material and Methods: This present cross-sectional study was carried out by recruiting 137 patients diagnosed of having liver abscess at medical OPD at JLN Hospital, Ajmer during the year December 2019 – June 2021. All the patients with confirmed liver abscess were included in this study. Patients age below 18 years, critically ill patient (Including patients having ruptured liver abscess at the time of presentation and requiring surgical intervention), Pregnancy and GI Malignancy

Results: 137 liver abscess patients were studied and males from lower middle class with a mean age of 38.41±8.81 years were more affected. Biochemical, serological, stool test, radiological and Mantoux test were performed and the mean alkaline phosphatase, GGT, SGOT, SGPT, bilirubin (Total/Direct/Indirect) and PT/INR were found to be raised.

Discussion: Right lobe of liver abscess was solitary. Right side pleural effusion followed by elevation of right hemidiaphragm was the abnormality diagnosed through X-ray. The survival rate was higher either with percutaneous needle drainage or pigtail drainage. E.coli followed by group A beta hemolytic streptococcus, tubercular and fungal etiologies were the most commonly isolated bacteria on pus culture. Blood, urine and stool culture showed positive cultures and some patients with tubercular liver abscess had mantoux test and sputum AFB positive.

Conclusion: Liver abscess is most commonly seen in male from lower middle class mostly in 4th decade of life who are non-vegetarian and alcoholic. As non-vegetarian diet and alcoholism were found to be the most common risk factors, it is suggestible to avoid/restrict their intake to avoid liver abscess.

Keywords: Liver abscess, usg, Entamoeba histolytica

Introduction

Liver abscess is an infective disease of parenchyma of liver, having collection of purulent material. The disease is caused by protozoal (amoebic), bacterial, mixed infections, tubercular and fungal infection. Among total cases of amoebiasis, around 3-9% cases are attributed to amoebic liver abscess[3]. Developing countries (eg. India) had endemicity for liver abscess because of overcrowding and poor sanitation.[1]

According to the previously available data, approximately more than 10% population throughout world hosts E. histolytica but according to the recent studies it is found that approximately 90% of cases are because of entamoeba dispar infection. It is around more than two decades, since the global magnitude to this disease was estimated[2,3]. Liver abscess had mortality rate in between 20-60%, along with proper surgical and conservative medical management.[4]

The potential routes for infection in liver parenchyma for occurrence of liver abscesses can be haematogenous route, ascend of infection from biliary tract or local spread from nearby infection sites.56 Older age groups incapacitated patients and in patients with malignancies are more prone for this disease.78

Pyogenic liver abscess is a infective disease of liver parenchyma having suppurative collection in it. It can develope as a complication of biliary tree diseases in around 40% of patients and have sustainably high mortality.[1] In recent times, the etiologies of Pyogenic liver abscess have shift from intra-abdominal source such as abdominal trauma and acute appendicitis to diseases of the biliary tree; but still, around 55% of patients do not have any apparent risk factors and hence , such cases are termed as cryptogenic.[9 10]

Therefore, we aimed to assess the clinical profile of patients and etiology of liver abscess.

Material and Methods:

This present cross-sectional study was carried out by recruiting 137 patients diagnosed of having liver abscess at medical OPD at JLN Hospital, Ajmer during the year December 2019 – June 2021. However, this study was initiated after the approval by ethical committee of this institute and obtaining consent from patients.

Inclusion criteria: All the patients with confirmed liver abscess were included in this study.

Exclusion criteria: Patients age below 18 years, critically ill patient (Including patients having ruptured liver abscess at the time of presentation and requiring surgical intervention), Pregnancy and GI Malignancy

All patients, after taking informed written consent were subjected to:

Blood investigations: Complete blood count, Total leucocyte count (/µl), Differential leucocyte count (%), Erythrocyte sedimentation rate (mm/hr), Total red blood cell count (/µl), Haemoglobin (g/dl), Total platelet count (/µl), Mean corpuscular volume (fL), Fasting Blood sugar (mg%), Random Blood sugar (mg%).

Liver function test: Serum total bilirubin (mg%), Direct bilirubin (mg%), Indirect bilirubin (mg%), SGOT (U/L), SGPT (U/L), Total protein (gm%), Serum albumin (gm%), Serum globulin (gm%), Alkaline phosphatase (U/L), Gamma glutamyl transpeptidase (U/L), PT (sec) /INR

Renal function test: Blood urea (mg%), Serum creatinine (mg%)

Electrolytes: Serum sodium (meq/L) and Serum potassium (meq/L)

Serology: IgG Anti Entamoeba Histolytica by card test, HIV test, HBs Ag test, Anti HCV

Other tests: C-reactive protein, Blood culture and Urine culture

Stool investigations: Stool culture for bacteria, Stool for trophozoite of Entamoeba Histolytica by immunochromatography

Radiological investigation: X-ray chest (PA view) and Ultrasonography (whole abdomen)

Mantoux test: Reference ranges of these laboratory parameters was define by the hospital laboratory's reference range.

All procedures were performed after correcting INR to < 1.4 in those patients who were having coagulopathy. We prefer pigtail catheterization in single, left lobe liver abscess, large (>10cm), partially liquefied abscess and abscess with thin rim.

Aspirate was collect in aseptic conditions in sterile containers and was sent to Department of microbiology immediately for:

1. Microscopic examination by wet mount for Entamoeba histolytica trophozoite.
2. Gram's staining.
3. ZN staining for Acid fast bacilli.
4. KOH Mount.
5. Bacteriological culture.

After the complete investigation, appropriate antibiotic/antitubercular drug / antifungal drug/anti amoebic drug will be started.

Statistical analysis: All data was collect in Microsoft-Excel sheet and was analysed with statistical software package SPSS version 26. Mean standard deviation and percentage was estimated.

Results:

Current study consists of 137 patients and found that 40.9% patients belong to the age group 40-50 years followed by 30-40 (29.9%) years, indicating that liver abscess is usually seen in 4th decade of life. Further, 83% of patients were males in the current study, indicating that males are commonly affected than females. Occupation wise, 30.6% patients with liver abscess were having private jobs, 25.5% labourers followed by 11.7% students. Driver, maids, house-wives were also there among participants. Further, 91.97% patients presented with fever followed by abdominal pain (75.91%) and other symptoms seen were nausea, vomiting, cough, diarrhoea, weight loss, chest pain, loss of appetite and pain in the right shoulder.

Table 1: Demographic variables of patients

Variables	Patients=137 (n %)	
Age wise distribution of liver abscess patient	<20	1(0.7%)
	20-30	29(21.2%)
	30-40	41(29.9%)
	40-50	56(40.9%)
	>50	10(7.3%)
Gender wise distribution of liver abscess patient	Female	22(16.1%)
	Male	115(83.9%)
Occupation wise distribution of liver abscess patient	Private job	42(30.6%)
	Labours	35(25.5%)
	Students	16(11.7%)
Presenting symptoms in liver abscess patients	Pain abdomen	104(75.91%)
	Fever	126(91.97%)
Risk factors for liver abscess	Non veg	82(59.85%)
	Alcoholic	78(56.93%)
	Smoker	67(48.90%)
	Tobacco chewer	54(39.42%)
	Diabetic	23(16.78%)
	RVD Positive	10(7.30%)
	History of malignancy	6(4.38%)

Distribution of alcoholism in patients of liver abscess	Non-alcoholic	59(43.1%)
	Occasional (≤3 times/ weeks)	37(27.0%)
	Regular (>3 times/ weeks)	41(29.9%)
Socio-economic status of patients of liver abscess	Lower Class	44(32.1%)
	Lower Middle Class	62(45.3%)
	Upper Middle Class	31(22.6%)

Table 2: Clinical variables of patients

Variables		**Patients=137 (n %)**	
Signs in patients of liver abscess on general physical and systemic examination		Hepatomegaly	92(67.15%)
		Guarding	88(64.24%)
		Icterus	37(27%)
		Pleural effusion	36(26.28%
		Oedema	32(23.35%)
		Pallor	15(10.94%)
		Lymphadenopathy	11(8.30%)
		Ascites	10(7.30%)
		Splenomegaly	5(3.64%)
		Rigidity	4(2.92%)
Complete blood count and coagulation profile in patients of liver abscess (Mean±SD)		Platelet Count	284.78±132.19
		MCV	90.06±10.24
		%Neutrophil	82.66±8.18
		ESR	37.35±21.66
		PT	24.33±8.15
		TLC	18.48±8.87
		Hb	11.14±1.70
		%Lymphocyte	8.11±5.48
		INR	2.0±0.68
Biochemical profile (Mean±SD)	Normal	Fasting sugar	(108.67±47.63)
		Random sugar	(158.16±71.84)
		Blood urea	(81.42±52.85)
		Serum creatinine	(1.32±0.66)
	Elevated	Total bilirubin	(3.50±2.48)
		SGOT	(178.21±89.18)
		SGPT	(190.85±93.01)
		Alkaline phosphatase	(143.47±83.98)
		Gamma glutamyl transferase	(55.13±31.80
Lobe of liver affected by liver abscess	Right lobe	87	63.5%
	Left lobe	32	23.36%
	Both lobes	18	13.14%
Number of liver abscess in patients	Single liver abscess	91	66.42%
	Few (≤3)	38	27.74%
	Multiple (>3)	8	5.84%
Chest X-ray	Normal	82	59.9%

	Right sided pleural effusion	24	17.5%
	Right sided hemidiaphragm elevation	21	15.3%
	Left-sided pleural effusion	1	0.7%
	Right sided and bilateral effusion	9	6.6%
Modality of abscess drainage in patients	Percutaneous Needle Drainage	99	72.30%
	Pigtail Drainage	38	27.70%
Management Outcome	Survived	122	89.1%
	Died	15	10.9%
Physical appearance of abscess fluid	Anchovy Sauce	81	59.1%
	Purulent	56	40.9%
Entamoeba histolytica serology (IgG)	Positive	71	51.8%
	Negative	66	48.2%
Wet mount for trophozoite	Positive	16	11.7%
	Negative	121	88.3%
AFB on pus abscess	Positive	9	6.6%
	Negative	128	93.4%
Gram staining of pus abscess fluid	Gram negative bacilli	30	21.9%
	Gram positive cocci	24	17.5%
	Negative	83	60.6%
KOH Mount on pus abscess fluid	Positive	1	0.7%
	Negative	136	99.3%
Abscess pus culture	Fungal culture on pus	1	0.73%
	Bacterial cultures on pus	51	37.2%
	Sterile	85	62.04%
Etiological bacterial agents	E Coli	19	13.89%
	Group A Beta haemolytic streptococcus.	18	13.14%
	Staphylococcus	5	3.65%
	Klebsiella	6	4.38%
	Enterococcus	4	2.92%
	Actinobacter	2	1.46%
	Pseudomonas	1	0.7%

	Citobacter	1	0.7%
Blood culture	Positive	30	21.9%
	Negative	107	78.1%
Urine culture	Positive	10	7.3%
	Sterile	127	92.7%
Bacterial Stool Culture	Normal Gut Flora	135	98.5%
	E. coli	2	1.5%
Detection of amoeba trophozoite in stool	Positive	10	7.3%
	Negative	127	92.7%
Mantoux Test	Positive	9	6.6%
	Negative	128	93.4%

Analysing the risk factors, 59.85% of patients having non-vegetarian diet had liver abscess and 56.93% patients were alcoholic including 29.9% patients were regular drinkers and 27% patients were occasional drinkers. 16.78% patients were diabetic. Other risk factors were smokers (48.90%), tobacco chewer (39.42%), RVD positive (7.30%), history of malignancy in past (4.38%). Socio-economic status of patients was also studied and found that 45.3% patients belonged to lower-middle class, followed by lower class, followed by upper-middle class, as per the modified Kuppuswamy's classification. Hepatomegaly (67.15%) was the most common sign followed by guarding (64.24%), followed by icterus, pleural effusion, edema, pallor, ascites, splenomegaly, and rigidity. The mean ESR was raised (37.35±21.66), indicating an associated inflammation in the body. Mean Neutrophils were (82.6%±8.18) and lymphocytes were (8%±5.48). INR and PT were raised among most of the patients with mean INR (2.0±0.68) and mean PT (24.33±8.15). Patients mean fasting sugar (108.67±47.63) and random sugar (158.16±71.84) levels, blood urea (81.42±52.85) and serum creatinine (1.32±0.66) were within normal range. But the mean Total bilirubin (3.50±2.48), SGOT (178.21±89.18), SGPT (190.85±93.01), alkaline phosphatase (143.47±83.98), gamma glutamyl transferase (55.13±31.80) were found to be elevated. Right lobe (63.5%) was most commonly involved followed by left lobe (23.36%) of the liver followed by both lobes of liver (13.14). 66.42% patients had a single liver abscess, while 27.74% patients had few (≤3) and 5.84% had multiple (>3) liver abscess.

Chest X ray was normal in 59.9% patients. Right sided pleural effusion was seen in 17.5% cases and right sided hemidiaphragm elevation was seen in 15% cases. Left-sided pleural effusion (0.7%) is very rare compared to right sided and bilateral effusion (6.6%). 72.3% cases were managed using percutaneous needle drainage. 27.7% cases were managed using pigtail drainage. Most of the patients (89.1%) had positive outcome (survived) while the mortality was seen in 10.9% patients

Anchovy sauce appearance of abscess fluid was seen in 59.1% cases and purulent appearance of abscess fluid was observed in 40.9% cases of liver abscess. 51.8% cases were having Entamoeba histolytica serology (IgG) positive indicating that they were having amoebic liver abscess. We mounted for trophozoite which was positive for 11.7% cases and negative for remaining cases. 6.6% of liver abscess patients were positive for acid fast bacilli in their pus. No organism was identified in 60.6% cases on gram staining of pus and rest of the cases showed gram negative bacilli (21.9%) compared to gram positive cocci (17.5%), on gram staining of pus. Only 1 patient (0.7%) showed budding yeast cells with pseudohyphae in abscess fluid on KOH mount. The pus of 62% patients was sterile with no organism on suitable

culture media, whereas, different bacterial flora were cultured in 37% cases, and in 0.73% patients fungal colonies are isolated from culture.

The most common bacterium that was isolated on pus culture was E Coli (13.89%) followed by group A Beta haemolytic streptococcus (13.14%). Staphylococcus (3.65%), Klebsiella (4.38%), enterococcus (2.92%), actinobacter (1.46%), pseudomonas (0.7%) and Citobacter (0.7%) were also isolated. 21.9% patients had positive blood culture for bacterial flora. Only 7.3% patients showed positive urine culture for bacterial flora. E. coli was isolated in only 1.5% patients in stool culture. In 7.3% patients with liver abscess, amoeba trophozoites were found in the stool sample. Mantoux test was positive in 6.6% patients. Acid fast bacillus was isolated in sputum by Zn-staining in 1.5% cases. CRP was positive in 78.1% cases.

Discussion:

This current study consists of 137 patients with liver abscess and patient's clinical examination was done in tertiary care centre at Central Rajasthan, North India during December 2019-June 2021.

The mean age of patients was 38.41years and most of the patients were males, indicating that liver abscess effects males compared to females and 32.1% belonged to lower socioeconomic status. In the study done by Soumikh Ghosh[1], it was found that mean age of patients with liver abscess as 41.13 years.

In the study done by Dhanapal[11], most of the patients with liver abscess belonged to 41-50 years age group, similar to our study. Shyam Mathur's 12tudied that most of the patients were males, similar to current study. In the study done by Vineet Jain,[13] the mean age of participants was 41.8 years and most of the subjects were males.

Alcoholism was found in many studies as one of the predisposing factors. 16.7% cases were having diabetes in the current study. 20% cases were having diabetes in Vineet's study. 6.7% were diabetic in Shyam's study. 4% patients were diabetic in Gubir Singh's study[14].

Right lobe was involved in most of the patients in the current study, Soumik's study[1], Dhanapal's study[2] and Shyam's study[3]. Both lobes were involved in 13% cases in the current study. Both lobes were involved in 15% patients out of 60 patients of liver abscess in Vandana Kumari's study[15]. 66.42% of cases had solitary liver abscess in the current study. 73% cases of Vandana's study had solitary lesions. 59% cases had solitary lesions in Naresh Pal's study[16]. 21-25% cases had multiple liver abscesses in the study by Sharma[17] done on Indian children. 5.8% patients had multiple liver abscesses in the current study done on Indian adults. 23% patients had multiple lesions in the study by Amit Ojha[18].

Amoebic liver abscess was seen in 51% cases in the current study. Most common organism isolated in the current study was E Coli (13.8%) followed by Group A beta haemolytic streptococcus. 8% cases had E Coli in the study done by Vandana.

In the current study, the most common symptom was fever followed by pain per abdomen. Nausea, vomiting and chest pain were also seen in some patients. In the study done by Shyam and Naresh Pal, right upper quadrant pain was the most common symptom.

Hepatomegaly followed by guarding was the most common signs seen in the current study. Hepatomegaly was seen in 29% patients in Naresh pal's study. It was 63% in Sharmila's study and 89% in Soumik's study and 86% in Vandana's study Pallor is the 2nd most common sign in Soumik's study.

INR and prothrombin time were deranged (increased) in the current study. INR (2±0.68) and PT (24.33±8.15) were deranged in 10% patients in Naresh Pal's study. INR was increased in 74% patients in Vineet's study.

ALP, SGOT, SGPT, gamma glutamyl transferase, total bilirubin was increased in many patients in the current study. Similar findings were seen in

Vineet's study. ALP was elevated in 82% cases in Vandana's study.

In the study by Sharmila, more patients with pyogenic liver abscess had increased total leukocyte count, bilirubin, SGOT and SGPT and alkaline phosphatase. In the current study, the differences in liver profile were not compared between amoebic and pyogenic liver abscess.

Conclusion:

Liver abscess is most commonly seen in male from lower middle class mostly in 4th decade of life who are non-vegetarian and alcoholic.

1. Most of the patients found to be having liver abscess were having coagulopathy (raised PT/INR) and most commonly isolated microorganism (bacteria) was E.coli.
2. The survival rate of patients with liver abscess was high because of use of percutaneous and pigtail drainage.

Consent

As per international standard or university standard, patient's consent has been collected and preserved by the authors.

Ethical Approval

As per international standard guideline written ethical approval has been collected and preserved by the author(s).

Competing Interest

Authors have declared that no competing interests exist.

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Bibliography:

1. Ghosh S, Sharma S, Gadpayle AK, Gupta HK, Mahajan RK, Sahoo R, et al. Clinical, laboratory, and management profile in patients of liver abscess from northern India. Journal Tropical Med. 2014;1:8.
2. Alam, F., Salam, M.A., Hassan, P. et al. Amebic liver abscess in northern region of Bangladesh: sociodemographic determinants and clinical outcomes. BMC Res Notes 7, 625 (2014). https://doi.org/10.1186/1756-0500-7-625
3. Walsh JA. Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. Rev Infect Dis. 1986 Mar-Apr;8(2):228-38. doi: 10.1093/clinids/8.2.228. PMID: 2871619.
4. NARWADE, Nandhkishore et al. What is the recent trend in the clinical study of liver abcess cases?. International Surgery Journal, [S.l.], v. 5, n. 4, p. 1382-1387, mar. 2018. ISSN 2349-2902. Available at: <https://www.ijsurgery.com/index.php/isj/article/view/2600>. Date accessed: 11 oct. 2022. doi:http://dx.doi.org/10.18203/2349-2902.isj20181115.
5. Conter RL, Pitt HA, Tompkins RK, Longmire WP Jr. Differentiation of pyogenic from amebic hepatic abscesses. Surg Gynecol Obstet. 1986;162:114-20.
6. Petri WA Jr, Singh U. Diagnosis and management of amebiasis. Clin Infect Dis. 1999;29:1117–25.
7. Serraino C, Elia C, Bracco C, Rinaldi G, Pomero F, Silvestri A, Melchio R, Fenoglio LM. Characteristics and management of pyogenic liver abscess: A European experience. Medicine (Baltimore). 2018 May;97(19):e0628. doi: 10.1097/MD.0000000000010628. PMID: 29742700; PMCID: PMC5959441.
8. Malik AA, Bari SU, Rouf KA, Wani KA. Pyogenic liver abscess: Changing patterns in approach. World J Gastrointest Surg. 2010 Dec 27;2(12):395-401. doi: 10.4240/wjgs.v2.i12.395. PMID: 21206721; PMCID: PMC3014521.
9. Kuo SH, Lee YT, Li CR, et al. Mortality in emergency department sepsis score as a prognostic indicator in patients with pyogenic liver abscess. Am J Emerg Med 2013;31:916–21. [PubMed] [Google Scholar]

10. Alvarez Pérez JA, González JJ, Baldonedo RF, Sanz L, Carreño G, Junco A, Rodríguez JI, Martínez MD, Jorge JI. Clinical course, treatment, and multivariate analysis of risk factors for pyogenic liver abscess. Am J Surg. 2001 Feb;181(2):177-86. doi: 10.1016/s0002-9610(00)00564-x. PMID: 11425062.
11. V., Dhanapal P.; R., Banurekha. Clinical profile of liver abscess in a tertiary referral hospital. International Surgery Journal, [S.l.], v. 4, n. 6, p. 2025-2029, may 2017. ISSN 2349-2902. Available at: <https://www.ijsurgery.com/index.php/isj/article/view/1259>. Date accessed: 11 oct. 2022. doi:http://dx.doi.org/10.18203/2349-2902.isj20172406.
12. Mathur S, Gehlot RS, Mohta A, Bhargava N. Clinical profile of amoebic liver abscess. J Indian Acad Clin Med. 2002;3:367-73.
13. JAIN, Vineet et al. Clinical and biochemical profile of liver abscess patients. International Journal of Research in Medical Sciences, [S.l.], v. 5, n. 6, p. 2596-2600, may 2017. ISSN 2320-6012. Available at: <https://www.msjonline.org/index.php/ijrms/article/view/3111>. Date accessed: 11 oct. 2022. doi:http://dx.doi.org/10.18203/2320-6012.ijrms20172454.
14. Singh G, Kumar R, Singh S, Kour G. A Study of Clinical Profile, Etiology, and Management of Liver Abscess in a Tertiary Care Center in Jammu. Int J Sci Stud 2020;8(6):71-74.
15. Vandana Kumari, Suman Kumar. A study of clinical profile and factors associated with the outcome in patients of dengue fever at tertiary health care centre. MedPulse International Journal of Medicine. August 2019; 11(2): 122-128. https://www.medpulse.in/Medicine/
16. @inproceedings{Pal2018LiverA, title={Liver abscess : clinical profile and management in tertiary care hospital}, author={Naresh Pal and Vineet V. Mishra and Nikhil Tayal and Vikram Singh}, year={2018} }
17. Sharma MP, Dasarathy, S, Amoebic liver abscess. Trop Gastroenterol 1993; 14:3.
18. Ojha, Amit, and Shaleen Tiwari. "Clinical profile and management of liver abscess by USG guided aspiration in combination with antibiotics and surgery: a clinical study." Journal of Evolution of Medical and Dental Sciences, vol. 3, no. 22, 2 June 2014, pp. 6105+. Gale OneFile: Health and Medicine, link.gale.com/apps/doc/A467680603/HRCA?u=anon~eb231fb6&sid=googleScholar&xid=62877073. Accessed 12 Oct. 2022.