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SERODIAGNOSIS OF AMOEBIC LIVER ABSCESS BY INDIRECT HAEMAGGLUTINATION TEST.

1. **AFTAB AHMED SOOMRO**
2. **JAWED AHMED BADVI**
3. **AMTUL HAFEEZ**
4. **AASIF DURANI**

1. ***Assistant Professor***
   Department of Pathology
   DOW MEDICAL COLLEGE, KARACHI.
2. ***Lecturer***
   Dept. of Pathology
   DOW MEDICAL COLLEGE, KARACHI.
3. ***Professor***
   LIAQUAT MEDICAL COLLEGE & DARUL SIHAT HOSPITAL, KARACHI.
4. ***Assistant Professor***
   Department of Pathology
   BMSI, JPMC, KARACHI.

Correspondence to:
AFTAB AHMED SOOMRO
Assistant Professor of Microbiology
Department of Pathology
DOW MEDICAL COLLEGE, KARACHI.

ABSTRACT

OBJECTIVE : *To assess the role of Indirect Haemagglutination Test for the diagnosis of amoebic liver abscess.*
STUDY DESIGN : *Comparative Study*
PLACE AND DURATION : *This study was carried out at Basic medical Sciences Institute, JPMC, Karachi from February 2000 to January 2001.*
SUBJECTS AND METHODS : *Blood samples were obtained from 100 cases of amoebic liver abscess and 100 age matched healthy individuals. Indirect haemagglutination test was performed on the serum to see the titres of antibodies to Entamoeba histolytica.*
RESULT : *Significant antibody titres ranging from 1 : 256 - 1 : 4096 were observed in confirmed cases of amoebic liver abscess. While titres were non-significant in normal subjects. IHA was found to be 96% sensitive and 100% specific for the serodiagnosis of amoebic liver abscess.*

KEY WORDS : Entamoeba histolytica, Hepatic amoebiasis, Indirect Haemagglutination (I H A) Test

INTRODUCTION

Amoebiasis is an endemic problem in many tropical and subtropical areas. World Health organization (WHO) has estimated that about 50,000,000 cases of invasive amoebiasis occur annually and it is among the leading causes of death due to parasitic infections surpassed only by Malaria and Schistosomiasis[1]. Amoebic liver abscess is the most common extraintestinal manifestation of invasive amoebiasis[2,3]. An immune dependant 170 KD surface protein of Entamoeba histolytica (EH) is considered to be essential for invasion of host tissue and it is responsible for resistance of the parasite towards hosts' immune response. This protein is recognized by antibodies in the sera of patients with amoebiasis, and this circulating antigen has been used as a diagnostic marker for amoebic liver abscess.[1] Serodiagnosis of amoebic liver abscess by Indirect Haemagglutination (IHA) test is considered to be Sensitive and Specific by many researchers.[4,5,6]
This study was designed to see the prevalence of amoebic liver abscess in Karachi with respect to socioeconomic status, living conditions, age and sex distribution, clinical signs and symptoms, radiological, and haematological laboratory findings and antiamoebic antibody titer level by haemagglutination (IHA) test.

SUBJECTS AND METHODS:

This study was conducted in the Department of Microbiology, Basic Medical Sciences Institute, JPMC, Karachi. A total of 100 cases of liver abscess were selected. The selection criteria was to include those cases who were clinically diagnosed for amoebic liver abscess and findings were confirmed on ultrasonography. Those cases that had hepatomegaly but were not diagnosed for amoebiasis clinically were excluded. For controls 100, age matched healthy individuals were selected. Hepatomegaly and amoebiasis was excluded in these cases on clinical assessment and ultrasonography. A sample of 5 ml blood collected from each case, serum was separated and samples were stored at -20º C, until tested for antibodies.
Indirect Haemagglutination test was run on the samples in batches, and for this purpose kit provided by Behring Diagnostic GMBH Marburg / Germany CELLOGNOST ® amoebiasis OTOMO G 1 7 C 20 (0927) H 1 Kit, (IHA) was used. According

to the test principle, specific antibodies cross link the sensitized erythrocytes in the reagent and form visible agglutinates. While in the absence of antibodies a button shaped deposit settles in the bottom of tube.[7]

Mean serum titers in infected cases range from 1:256 to 1:2048, while in the case of healed lesions low titers of 1:32 were observed. Titers of 1:128 are considered to be borderline and were repeated, while titers below 1:32 exclude invasive amoebiasis.[5] All the 200 samples were subjected to IHA test and results were recorded. The serum antibody titers, in clinically established cases and controls were compared and the sensitivity and specificity of test was calculated.

TABLE 1.
Socio economic status of amoebic abscess patients

INCOME			JOB		EDUCATION		TYPE OF AREA		
A	B	C	Office	Manual	Yes	No	Slum	Semi urban	Urban
45	44	11	44	56	70	30	48	29	23
45%	44%	11%	44%	56%	70%	30%	48%	29%	23%

TABLE 2.
Living conditions of amoebic abscess patients

HOUSE		WATER SUPPLY				TOILET	
Pacca	Kacha	A	B	C	D	Flush system	Pit system
56	44	56	20	12	12	75	25
56%	44%	56%	20%	12%	12%	75%	25%

TABLE 2.
Living conditions of amoebic abscess patients
graphic representation

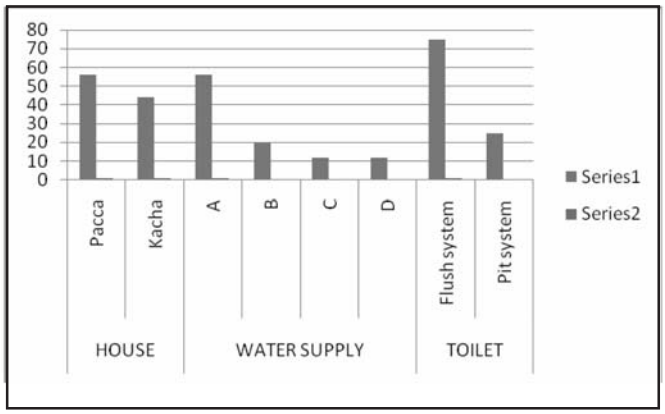
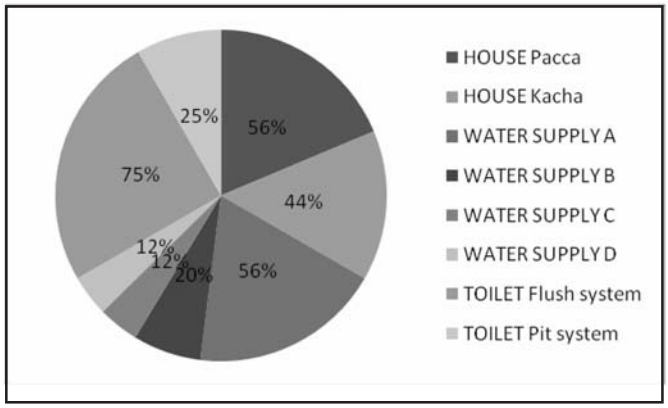


TABLE 2.
Living conditions of amoebic abscess patients
pie-graphic representation



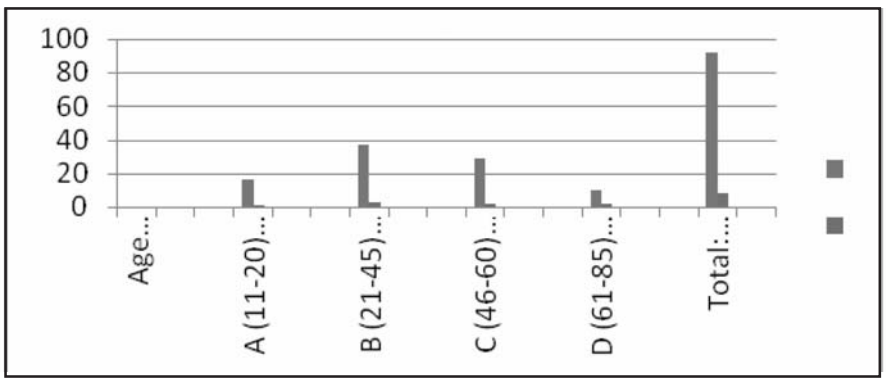
RESULTS:

(Table 1) shows socioeconomic status of patients diagnosed amoebic for amoebic liver abscess. This included the total income, nature of job, education and type of residence where they lived. The group A included poor people and they were 45%. The group B included as middles class and they were 44%. The group C included the peoples above middle class and they were 11%. Forty four percent patients or their parents were workers and fifty six percent patients or their parents were manual workers. Seventy percent had education at primary level or above while the rest were illiterate. Majority of the patients (48) came from slum area, 29% came from semi urban area and only 23% from urban community.

(Table 2) shows living conditions of amoebic liver abscess patients as regards water supply and type of toilet. Fifty six percent had supply of water by water board (A), 20 % used the water supplied from community tap (B), 12% used the water supplied by

Table 3:
AGE AND SEX DISTRIBUTION OF AMOEBIC LIVER ABSCESS PATIENTS.

Age Group (Years)	Male (%)	Female (%)
A (11-20) n=17	16	1
B (21-45) n=40	37	3
C (46-60) n=31	29	2
D (61-85) n=12	10	2
Total: n=100	92	8



water tankers or carriers (C) and supply, and 74% had used water from more than one source. Regarding the type of toilet, 75% had flush system and 25% had pit latrine.

Table 3 shows age and sex distribution of amoebic liver abscess patients. They were divided in four groups. Group A included teenagers, age rangingfrom11 to 20 years. They were 17%, (male 16% and female 1%). Group B included young persons of 20 – 45 years and they were 40%, (male 37% and female 3%). Group C comprised of persons 46 to 60 years of age and they were 31%, (male 29% and female 2%). Group D comprised of old persons having more than 60 years of age. They were 12%, (male 10% and female 2%).

Clinical features were suggestive of amoebic liver abscess as shown in (Table 4). In these cases Fever, Pain in right hypochondrium, tenderness and hepatomegaly was the most common feature followed by anorexia and weight loss.

Table No.4.
Signs and Symptoms on clinical examination in case of liver abscess.

SIGNS AND SYMPTOMS	NUMBER OF CASES (%)
Abdominal pain	95
Fever	95
Liver tenderness	95
Hepatomegaly	95
Anorexia	56
Malaise	48
Weight loss	45
Chills and rigors	30
Dyspnea	25
Diarrhoea	19
Cough	13
Jaundice	13
Spleenomegaly	03

Table No.4.
Signs and Symptoms on clinical examination in case of liver abscess ***graphic representation***

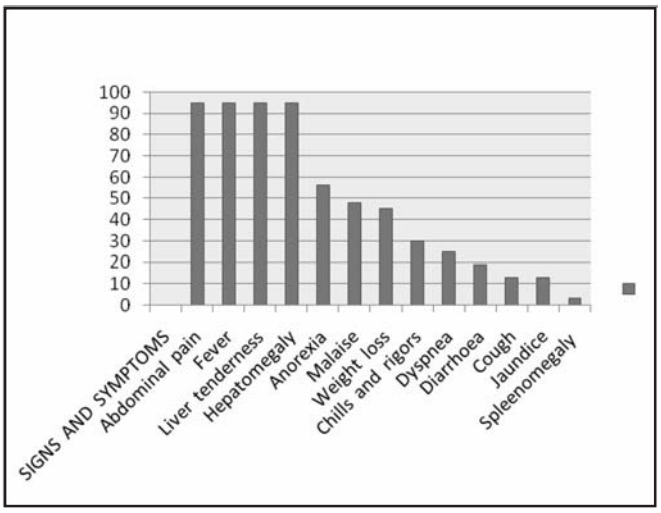
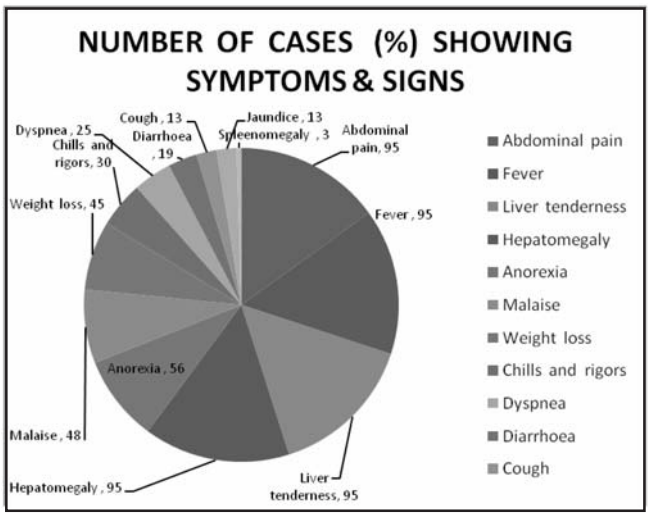


Table No.4.
Signs and Symptoms on clinical examination in case of liver abscess ***piegraphic representation***



(Table 5) revealed the X-Ray chest PA view findings, somewhat similar results from Pakistan[9] and Taiwan[15].

Table 6. show the ultrasonographic findings suggestive of liver abscess. The most prominent feature being the involvement of right lobe of liver in (80 %) cases.

Table 7. shows the laboratory result of amoebic liver abscess patients. WBC count was slightly increased (11000- 25000/cmm) in 70% of patients. ESR was raised in 48% of cases. Haemoglobin < 10 gm% in 10% patients. Raised alkaline phosphatase > 90 IU/L was present in 44% cases, ALT > 40 IU/L in 30% of patients while total bilirubin was raised > 2mg% in 13%.

The antibody titers on IHA test in cases of amoebic liver abscess are shown in (Table 8). The titers indicate significant levels in 96 cases, while in 4 cases the titers were found to be below 1:256. These were considered as borderline and when repeated, showed lower titers. According to the observations and results of this study IHA test was found to be 100 % Specific and 96% Sensitive. The positive predictive accuracy was found to be 100 %.

DISCUSSION

This study was designed to see the prevalence of amoebic liver abscess in Karachi with respect to socioeconomic status, living conditions, age and sex distribution, clinical signs and symptoms, radiological, and haematological laboratory findings and antiamoebic titer level by haemagglutination (IHA) test.

General information in each case revealed that the most of the patients were either from low income or middle class families. Majority of the patients were manual workers (Table 1) having some education at lower or higher secondary level. Patients come from all the five districts of Karachi because JPMC is one of the biggest hospital of this city . Some patients belonged to the suburbs of Karachi and of slum areas there were also a number of patients who came from interior of Sindh province. In the present study majority of patients were residents of slum areas (Tab. 1) which are

known to harbor many microbial agents due to over crowding poor sanitation, unhygienic environment, polluted water and contaminated food supplies.

(Tab. 2) elaborates the water sources type of houses and toilets of the selected population, in majority of the cases due to over crowding poor sanitation, unhygienic environment, polluted water and contaminated food supplies, all this comes in predisposing conditions. Western countries have also recently experienced a resurgence of amoebic liver abscess associated with immigrants, overcrowding and reduced living standards[6].

In (Tab. 3) it is shown that males were found to be more affected than females. Similar pattern was also seen in Iraq[13], in Pakistan[9] in Japan[16], India etc. it was noted that the amoebic liver abscess had occurred in all age groups in the present study, more or less equally, but the prevalence rate was higher in the age group between 20-45 years. In (Tab. 4) patient´s history and physical examination revealed that fever, right upper abdominal pain, hepatic tenderness and hepatomegaly were present in 95%. The other symptoms were anorexia chills, weight loss, malaise, dyspnea, diarrhea, cough and slight speeleenomegally. Similar results were obtained with little difference from Pakistan[9&6], from iraq[13], Taiwan[15] and India.

(Tab. 5) revealed the X-Ray chest PA view findings, somewhat similar results from Pakistan[9,] and Taiwan[15].

(Tab. 6) show the ultrasonographic findings suggestive of liver abscess. The most prominent feature being the involvement of right lobe of liver(80 %) cases. More or less these findings mimic with others studies from Pakistan. [9,14,] and Taiwan[15]. The laboratory investigations in amoebic liver abscess patients are shown in (Tab. 7) with some variations from Pakistan[9,14,15].

Serum titers of antiamoebic antibodies in different age groups are shown in (Tab. 8). The titers indicate significant levels in 96 cases, while in 4 cases the titers were found to be below 1:256. These were considered as borderline and when repeated, showed lower titers. According to the observations and results of this study IHA test was found to be 100 % Specific and 96 %

Table No.5
Radiographic findings of amoebic liver abscess patiens

X-RAY CHEST	PERCENT OF PATIENTS
Right hemidiaphragm elevation	40%
Right pleural effusion	05%
Normal	55%

Table No.5.
Radiographic findings of amoebic of liver abscess ***graphic representation***

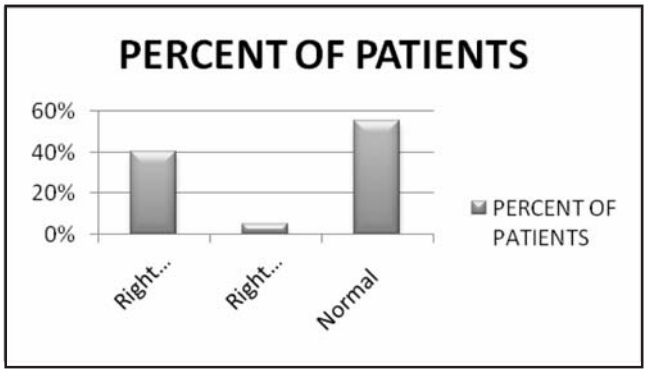


Table No.5.
Radiographic findings of amoebic of liver abscess ***pie-graphic representation***

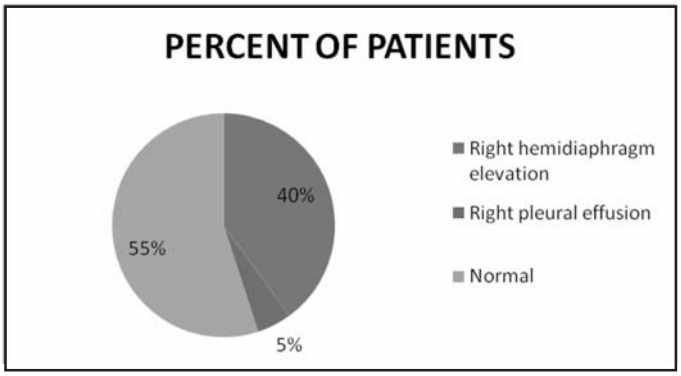


Table No.6.
Size and Location of amoebic of liver abscess on ultrasonography

Size	Mean size (cm)	Percent of Patients
	> 5 cm	30 %
	= 5 cm	70 %
Location	Right Lobe	81 %
	Single	88%
	Multiple	12 %
	Left Lobe	14 %
	Both Lobes	05 %

TABLE No. 7
Abnormal laboratory results of 100 patients with amoebic liver abscess

LABORATORY RESULTS	PERCENT OF PATIENTS
White blood cell counts (>11,000 – 25,000/mm³)	70
ESR > 10 mm	48
Haemoglobin < 10g %	10
Alkaline posphatase > 90 U/L	44
ALT > 40 U/L	30
Total bilirubin > 2mg %	13

TABLE No. 7-
Abnormal laboratory results of 100 patients with amoebic liver abscess ***pie-graphic resentation***

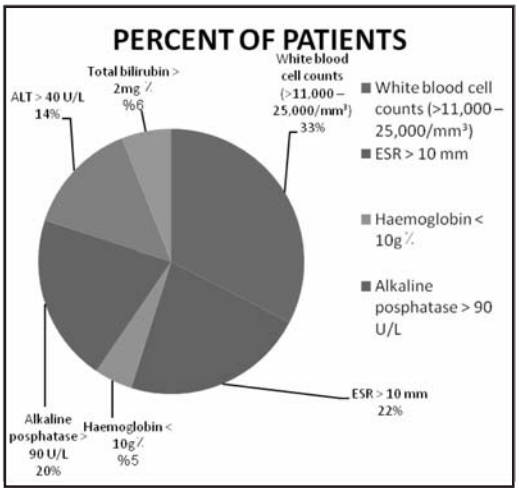
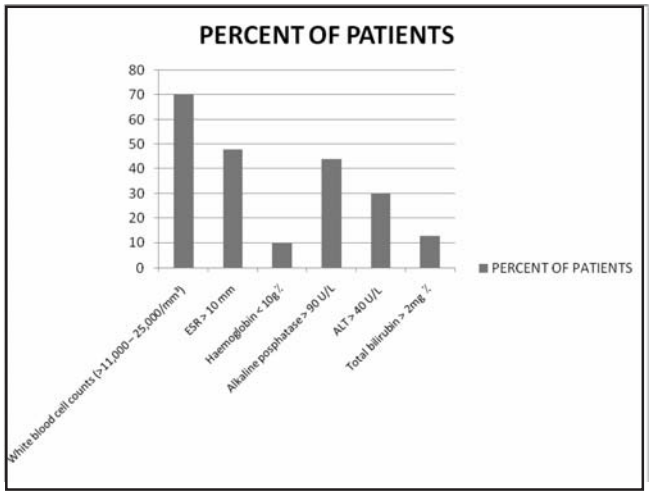


TABLE No. 7-
Abnormal laboratory results of 100 patients with amoebic liver abscess ***pie-graphic resentation***



Sensitive.
The positive predictive accuracy was found to be 100 %.

CONCLUSION

In the light of results of this study it can be suggested that IHA still remains a reliable, economic and easily performed diagnostic tool which can be recommended for serodiagnosis of amoebic liver abscess. It is concluded that IHA test is a sensitive and specific test for diagnosis of amoebic liver abscess.

TABLE No. 8 -
IHA Serum titers in patients of amoebic liver abscess

Age Group/ Level of titer	1:128	1:256	1:512	1:1024	1:2048	1:4026
A (11-20) n=17	1	0	8	5	2	1
B (21-45) n=40	2	2	15	12	7	2
C (46-60) n=31	1	1	13	10	4	2
D (61-85) n=12	0	0	6	4	2	0
Total	4	3	42	31	15	5

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