

Serological studies of *Entamoeba histolytica* infection in north Indian population

M.P. Sharma, B.M. Gandhi, S.K. Acharya, Y.K. Chawla
B.L. Nautiyal & B.N. Tandon

Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi

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The per cent rate of antibody to *E. histolytica* in different socio-economic groups of north Indian adult population was evaluated by the indirect haemagglutination assay (IHA) and counterimmuno-electrophoresis (CIEP). The overall positivity rate of antibody to *E. histolytica* in these groups was 1.01 per cent by IHA and 0.52 per cent by CIEP. A study of alcoholics, diabetes mellitus, healthy factory workers, Central Reserve Police Force jawans, healthy tribal population and voluntary blood donors, revealed a high antibody positivity rate in alcoholics, as compared to others. Diabetes mellitus, mild malnutrition and strenuous physical work did not increase the positivity rate for antibody to *E. histolytica*.

A small proportion (about 3—9%) of patients carrying cyst of amoeba in their colon develop amoebic liver abscess¹. The reason why some individuals develop amoebic liver abscess (ALA), while others do not is not known. It has been suggested that several host factors predispose to ALA, such as a pre-existing liver damage specially due to alcohol, diabetes (N. Madanagopalan, personal communication) or malnutrition², besides the virulence of the parasite³ and the immunity of the host⁴.

Invasion of target cells by *Entamoeba histolytica* produces humoral response and specific antibody can be detected in the serum a week after infection³. Highest amoebic antibody titres are found in acute invasive amoebiasis⁵. Prevalence of invasive amoebiasis in a given population

can be studied by several approaches viz., clinical⁶, ultrasound imaging⁷ and amoebic serology⁸⁻¹⁰. Since antibodies to *E. histolytica* persist in serum for a few years after invasive amebiasis¹¹, serological investigation would be the simplest approach to determine the prevalence of invasive amoebiasis in the community. The frequency of antibody to *E. histolytica* has been shown to vary from 2 to 18 per cent in adults of apparently healthy population¹²⁻¹⁴.

A number of serologic tests are available to detect antibodies to *E. histolytica*. Indirect haemagglutination assay (IHA) is the technique of choice for epidemiological studies, because of its higher sensitivity and specificity¹⁵⁻¹⁷.

The rate of antibody to *E. histolytica* in different socio-economic group of

adults in north Indian population is reported here and analysed to find out the association of a few host factors like alcoholism, diabetes, and socio-economic and environmental factors with the *E. histolytica* antibody positivity rate.

Material & Methods

The following groups of subjects (total 1806) were selected for serological studies for amoebic antibody *viz.*, alcoholics (68), patients of diabetes mellitus (434), healthy factory workers (85), CRPF jawans (580), healthy tribal population (108) and voluntary blood donors (530).

Alcoholics : This group included young truck drivers. In India, there are professional private companies which run the truck services and each company maintains around 500 to 1000 trucks and employs drivers on a regular salary. The number of drivers is invariably less than the number of trucks pressed into service. The drivers are thus forced to work overtime. Each truck driver usually works for 100 to 120 h a week and turn to alcohol for relief of fatigue. Our history revealed that they consumed 450 to 500 ml of whisky or rum every day. Their total caloric intake was 3500 to 4000 calories per day. Living conditions of the drivers were unhygienic, in crowded areas of the city where sanitation is poor. High illiteracy and poor personal hygiene were also common among the truck drivers. Two negative host factors *viz.*, alcohol and poor sanitation were thus present in this group.

Diabetics : Only those who were clinically and biochemically overt diabetics, and attending the Diabetic Clinic at the All India Institute of Medical Sciences,

New Delhi were included in this group. This group was included because (i) diabetics are more prone to infection and tissue invasion; (ii) their total calorie intake being invariably restricted, under-nutrition is common in them; and (iii) a high proportion of diabetics are known to get a fatty liver. These three factors may increase the chances of hepatic invasion by *E. histolytica*.

Healthy factory workers : Subjects working in a pharmaceutical company, in a suburban state 30-45 km from Delhi were included in this group. Most of the workers were from local semi-urban area and belonged to the low socio-economic class. These workers were exposed to an industrial environment and heavy physical work for the past 10 to 12 yr. They lived in a poor sanitary condition, although their clinical nutritional status was good.

CRPF jawans : The Central Reserve Police Force is a paramilitary force in our country. Soldiers were selected from this group because (i) men from different parts of the country are recruited to the army and thus they represent a good sample of the country; (ii) their living conditions are good, as the Government provides them decent quarters, safe and adequate water and good sanitary conditions; (iii) their nutritional status is excellent as they get a balanced diet with 3500 to 4000 calories per day; and (iv) the soldiers are subjected to a regular medical checkup.

Tribal population : This group of population belonging to Palaman district of Bihar was included because (i) the tribals live in interior parts of the rural and forest regions about 100 km away from modern civilization ; (ii) they drink water from wells and fresh streams; and (iii)

their environment is not polluted with sewage, flies, decomposed garbage *etc.*

Voluntary blood donors : This group included men and women belonging to middle or high socio-economic status. The group provided a homogeneous sample of the Delhi population.

Technique : Blood samples were collected from different groups of population and the serum separated and kept at -75°C till the time test was carried out. Counter immunoelectrophoresis (CIEP) for detection of amoebic antibody was done by the modified method of Krupp¹⁸. The detection of amoebic antibody by CIEP was used as the first screening procedure. Indirect haemagglutination assay (IHA) was carried out by the method of Prakash¹⁹. The detection of the titres of amoebic

antibody by IHA was used as the second screening procedure.

Results & Discussion

Table I shows the rate of amoebic antibody in different groups of population. The data are presented in terms of positive percentage both by IHA and CIEP (Table I) and IHA titre (Table II). A sample was labelled as positive by IHA if it had a titre of 1 : 162 or above¹⁵.

None of the samples in alcoholics was positive by CIEP. Out of 68 samples tested, 3 (4.5%) showed a titre of 1 : 162 and above with IHA.

In diabetes mellitus two samples showed the presence of amoebic antibody out of 434 samples tested with CIEP (0.5%) as compared to 0.9 per cent with IHA, where 3 samples were positive of 326

Table I. Prevalence of amoebic antibody in different groups of population

Group	CIEP		IHA	
	No.	No. positive	No.	No. positive*
Alcoholics	68	0	68	3 (4.5)
Diabetes mellitus	434	2 (0.5)	326	3 (0.9)
Healthy factory workers	86	0	83	0
CRPF jawans	580	7 (1.2)	468	6 (1.3)
Tribal population	108	0	108	0
Voluntary donors	470	0	530	4 (0.8)
Total	1746	9 (0.52)	1583	16 (1.01)

Figures in parentheses indicate the percentage; *IHA positive—titre \geq 162

Table II. IHA titre of amoebic antibody in different groups of population

Group	Total no.	No. of IHA positive	
		<1 : 162	≥1 : 162
Alcoholics	68	12 (15.4)	3 (4.5)
Diabetes mellitus	326	52 (15.9)	3 (0.9)
Healthy factory workers	83	6 (7.2)	0 (0.0)
CRPF jawans	468	139 (29.7)	6 (1.3)
Tribal population	108	6 (5.6)	0 (0.0)
Voluntary donors	530	177 (33.4)	4 (0.8)
Total	1583	392 (24.8)	16 (1.01)

Figures in parentheses represent percentages

samples tested. Among factory workers none of the 86 samples showed positive reaction either by CIEP or IHA.

Among CRPF Jawans, 7 (1.2%) of the 580 samples tested showed positive reaction by CIEP whereas 468 samples were tested, for amoebic antibody by IHA technique and 6 (1.3%) showed positive reaction.

Out of 108 blood samples from tribal population tested, none gave positive results with either CIEP or IHA.

Among voluntary donors, CIEP was positive in none of the blood samples and IHA was positive in 4 out of 530 samples. The positive percentage was 0.8 per cent with IHA.

The overall positivity rate in all these groups combined together was 0.52 per cent (9 out of 1746 blood samples) with CIEP and 1.01 per cent (16 out of 1583 blood sample) with IHA.

The results of serological studies for invasive amoebiasis in different groups of population included in the present study shows highest *E. histolytica* antibody positivity rate amongs alcoholic which would suggest that alcoholic liver injury may pre-dispose to invasive form of amoebic disease. 4.5 per cent of the alcoholic truck drivers regularly consuming more than 200 g of absolute alcohol per day had amoebic antibody by IHA test in titres equal to or above 1 : 162. The clinical association of amoebic liver abscess with alcoholism has been reported from India and South Africa¹. The latter report emphasises that (i) often patients of liver abscess give history of significant consumption of alcohol preceding the onset of illness and (ii) a decline in the incidence of liver abscess reported during the periods of prohibition followed by an increase again when the rules were relaxed for consumption of alcohol. The exact mechanism by which alcohol pre-disposes to invasive amoebiasis is not known.

Diabetes mellitus predisposes individuals to bacterial infections. High proportion of diabetics have significant fatty infiltration of liver. In the present series of 326 patients of diabetes mellitus, only 3 gave positive serological test for amoebiasis, suggesting that diabetic mellitus does not contribute to invasive amoebic disease to any significant extent.

The tribal population studied was exposed to mild nutritional deficiencies particularly calories, proteins and vitamin 'A' but their environment was quite healthy. The drinking water supply was limited but usually clean. The factory workers studied in the present series, lived in semi-urban slum environment

and consumed food unprotected from dust and flies, but their nutritional status was good. Neither of these two groups showed evidence of invasive amoebiasis by serological tests, suggesting that mild nutritional deficiencies and minor aberrations of environment do not lead to increased incidence of invasive amoebiasis. However, this does not minimise the role of moderate and severe malnutrition or a too bad personal and environmental hygiene in epidemiology of invasive amoebiasis. Even in countries with endemic *E. histolytica* infection in colon, large sections of the community belong to the former category and only a small proportion is exposed to the hazards of highly unfavourable nutritional status and very poor environmental and hygienic conditions.

Voluntary blood donors and CRPF jawans were the best amongst all the groups studied, with respect to nutrition, personal hygiene and environment. Their socio-economic status and pattern of living promoted alcohol intake through 'social drinking'. However, they were not regular alcoholics. The CRPF jawans had more opportunities for getting alcohol at a low cost than did the voluntary donors and perhaps drank more frequently. Serological evidence of invasive amoebiasis in these two groups was positive in 0.8 and 1.3 per cent respectively. Alcohol may have same role to play in this positivity rate specially in CRPF jawans.

Our study of different groups of individuals thus suggests that alcohol may predispose to invasive amoebiasis. Diabetes mellitus, mild malnutrition, minor environmental hazards and strenuous physical work, on the other hand did not

increase the chances of invasive amoebiasis. Serology positivity rate in Mexico¹² has been reported to be 6 per cent as compared to 1.01 per cent in the present report. Both the countries are endemic for *E. histolytica* infection of the colon. There is a possibility that alcohol consumption, which is more frequent in the western society than in India, has some relationship in view of the nearly 6 times higher prevalence of invasive amoebiasis in Mexico as compared to India.

The significance of the detection of amoebic antibody in such low titre as 1 : 18, 1 : 54 by IHA is not known. It may be an indication of a minor invasion of colon in the past. If 1 : 54 titre is considered to be of significance in this respect, in our study (Table II) except for the tribal population and healthy factory workers, a significant proportion of other groups varying from 5 to 33 per cent had suffered with invasive amoebiasis sometime in the past. A study on a larger sample of population may help in determining significance of low IHA in the community.

Our study shows that counterimmunoelectrophoresis test (CIEP) is a simple and quick test for detection of amoebic antibody but its sensitivity for detection of amoebic antibody is low in comparison to indirect haemagglutination test.

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Reprint requests : Dr B.N. Tandon, Professor of Medicine, Department of Gastroenterology and Human Nutrition Unit, All India Institute of Medical Sciences, Ansari Nagar New Delhi 110029