

# Invasive Amoebiasis: Circulating Antibody Levels by Latex Agglutination Test\*

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## SUMMARY

Sera from 97% of 200 patients with invasive amoebiasis and 15% of 100 general medical patients gave positive results when quantitatively tested with a latex agglutination test. All degrees of agglutination were observed and no correlation was found between latex agglutination results and non-invasive amoebae in the gut. Follow-up sera obtained over a period of 6 months from 8 patients showed reactions of varying intensity. It was concluded that individual variation in antibody production was such that the test could not be used as an index of severity but should simply be read as either positive or negative.

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*Entamoeba histolytica* is an organism which gives rise to easily detectable specific antibody when human tissues are invaded and several serological tests have thus been devised. Most are capable of quantitation by dilution of the serum to the point at which antibody activity is no longer detectable and the titre thus obtained reflects the level of circulating antibody in the subject being tested. A range of antibody levels is observed in any population where invasive amoebiasis is endemic.<sup>1</sup> Queries arising from previous publications<sup>2,3</sup> and experimental material provided to workers in other parts of the world suggest that a low titre often raises doubts when making a differential diagnosis as one is tempted to correlate the intensity of the observed reaction with the severity of the disease being considered.

This study seeks to show the relationship between the various stages of invasive amoebiasis, both before and

after treatment, and the quantitative results of the amoebic latex agglutination test.

## MATERIALS AND METHODS

Three hundred sera were examined from adult Bantu hospital patients in an area where *Entamoeba histolytica* is endemic. Those with proved invasive amoebiasis were classified as amoebic liver abscess (100) where characteristic pus was aspirated and as amoebic dysentery (100) where haematophagous trophozoites were found in either dysenteric stools or rectal scrapings. Specific response to treatment with metronidazole was subsequently observed in these 2 groups. A control group of general medical patients (100) showing no evidence of invasive amoebiasis, was also examined for intestinal amoebae by direct microscopic examination of stool smears and by the zinc sulphate flotation technique. All the observed amoebae were identified as to species. Sera were selected for the study according to clinical criteria as specimens were submitted for routine diagnosis on admission of the patients. In addition, sera were obtained from 8 of the proved invasive amoebiasis patients at irregular intervals over a period of 6 months.

An amoebic latex agglutination test \* was performed on each serum after it had been heated to 56°C in a waterbath for 20 min. Sera were tested in groups of 5 on a glass plate ruled into 5 rows of 5 squares, each approximately 2 × 2 cm. One drop of sensitized latex was placed in each square of the first row by touching the glass with the pipette rather than by letting the drop fall free. An equal-sized drop of serum was placed next to each drop of latex and the drops in each square were then thoroughly mixed by means of a piece of orange stick. The plate was then gently rocked by hand for 5 minutes. Positive results were recorded at 1-minute intervals from the time of mixing. Reactions were thus rated as 4+ where agglutination was obvious for 4 minutes of the testing time, and similarly 3+, 2+, 1+ and finally 0 where no agglutination was visible at the end of the 5 minutes. The essential feature of the 0 or negative reaction was that the drop remained opaque and homogeneous, whereas in the 1+ reaction a rim of agglutinated particles was clearly seen. The next row of 5 tests was then prepared and read.

A series of serial two-fold dilutions of serum which showed 4+ reactions from 3 patients with amoebic liver abscess was also tested.

## RESULTS

Each kit was found to provide sufficient material to test at least 25 sera. The types of reaction observed are illustrated in Fig. 1.

Table I shows the effect of dilution of strongly reactive sera on reaction intensity and thus confirms that the rating system adopted does reflect the relative concentration of antibody in the sera tested.

Fig. 2 shows that reaction intensities covering the whole range were observed in the undiluted sera of each group, including the control group of general medical patients in



0 1+ 2+ 3+ 4+

Fig. 1. Reaction intensities observed with whole sera in amoebiasis latex agglutination tests. Note particularly that 0 or negative reaction is opaque and homogeneous.

TABLE I. INTENSITY OF REACTION WITH SERIAL DILUTIONS OF SERA FROM 3 PATIENTS WITH AMOEBIC LIVER ABSCESS

Patient	Dilutions						
	1:2	1:4	1:8	1:16	1:32	1:64	
A	...	4+	3+	2+	1+	0	0
B	...	3+	2+	1+	0	0	0
C	...	4+	3+	3+	2+	1+	0

which only 15% were positive. Within the latter group, 30 patients were found to be harbouring intestinal amoebae, identified as *Iodamoeba butschlii*, *Endolimax nana*, *E. coli*, *E. hartmanni* and *E. histolytica*, either singly, or in various combinations. Table II shows an analysis of the control group.

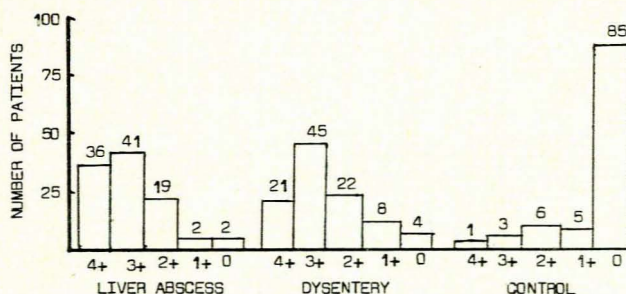


Fig. 2. Distribution of reaction intensity of whole sera used in amoebiasis latex agglutination tests. Note trends indicated by comparing 0 and 4+ categories between groups.

Table III shows that considerable individual variation is apparent in the results obtained with sera from the 8 patients taken up to 6 months after admission; although in 5 cases, circulating antibody fell to undetectable levels within 3 months of treatment.

It is important to note that all categories of patients showed reactions covering the whole range of intensities with 2% of amoebic liver abscess and 4% of amoebic

\*Material available in kit form from Miles-Serovac (Pty) Ltd, P.O. Box 141, Goodwood, CP.

TABLE II. MICROSCOPIC STOOL EXAMINATION AND LATEX AGGLUTINATION TEST RESULTS ON 100 GENERAL MEDICAL PATIENTS

Intestinal amoebae	Agglutination	
	+ <sup>ve</sup>	- <sup>ve</sup>
<i>E. histolytica</i>	0	3
Other	3	24
None	12	58
	—	—
	15	85

TABLE III. REACTION INTENSITY OF FOLLOW-UP SERA FROM 8 PATIENTS WITH PROVED AMOEBIASIS ON ADMISSION

Patient	Time in months						
	0	1	2	3	4	5	6
A	0	0			0		0
B	1+	1+	2+		3+		3+
C	3+	3+		4+			4+
D	2+	0		0		0	
E	3+		0		0		0
F	3+	3+		0			0
G	3+	2+		0		0	
H	4+	2+				0	

dysentery negative, while 4% of the control group were strongly positive.

## DISCUSSION

The results obtained in this study are compatible with the concept that parenteral contact with a parasite is necessary to initiate the cellular response which gives rise to circulating antibody.

In the case of amoebiasis, the amoebae initially live in a non-invasive state in the lumen of the gut. This stage was represented by the 3 patients in the control group with purely intestinal *E. histolytica*, none of whom showed

detectable circulating antibody. The next phase, which may be manifested as amoebic dysentery, involves invasion of the gut wall and the test was positive for 96% of these patients. Formation of an amoebic liver abscess may follow as the third stage of the invasive process and 98% of this group showed detectable antibody. The over-all 97% positive result with invasive amoebiasis indicates a strong correlation between invasion and detectable circulating antibody. No corresponding reaction to species of non-invasive amoebae in the lumen of the gut was seen as only 3 of the 30 patients in the control group in this category gave positive reactions. One may speculate that the antibody in the control group as a whole arose from subclinical infections or else it may have been residual after previous infections and as such should not be considered as 'false positive'. This consideration applies to all serological tests based upon the detection of circulating antibody.

The distribution within the groups in Fig. 2 shows that, considered as a whole, the patients with liver abscess tend to produce slightly more antibody than those with amoebic dysentery, and that the control group produced significantly less. Ranges overlap to such an extent, however, that a single quantitative observation cannot be related to the state of the disease in any particular individual. This is quite understandable as many factors influence the observed antibody level. Among the most important are the duration, degree and site of the infection and the efficiency of the antibody generating system of the individual concerned. There was no means of assessing these parameters in naturally infected human patients either before or after treatment and no provision could be made for the intermediate and overlapping stages which must have been encountered in a sample of this size. Attempts to interpret the reaction on a quantitative basis as an index of the degree of invasion can only lead to confusion.

One may conclude that the latex agglutination test for invasive amoebiasis should be read simply as either positive or negative.

## REFERENCES

1. Krupp, I. and Powell, S. J. (1971): *Amer. J. Trop. Med. Hyg.*, **20**, 414.
2. Morris, M. N., Powell, S. J. and Elsdon-Dew, R. (1970): *S. Afr. Med. J.*, **44**, 594.
3. *Idem* (1970): *Lancet*, **1**, 1362.