STUDIES ON GELL DIFFUSION TEST IN AMEBIASIS

Aly Khan and Bilqees Mujib

Abstract

Serological diagnosis of amebiasis by Gell-diffusion tests, were conducted on 39 patients during a period of 8 months. Twenty eight patients were from Medical Ward II, Civil Hospital, Karachi, while the rest were volunteers suffering from dysentery. Among these 8 had living trophozoites of ENTAMOEBA HISTOLYTICA in the stool, but by Gell-diffusion test we were able to confirm that 24 patients had amebiasis because these gave positive results repeatedly and were also suspected for this disease by the doctors (JPMA 29:268, 1979).

Introduction

Gell-diffusion tests have been widely used during the recent years in the study of epidemiology of disease, monitor human pathology and physiology and can be used whenever antigen or antibody are needed to be quantitated or characterized. By Gell-diffusion test we can identify antigen-antibody reactions through its coalescence with a reference line of precipitation. These tests are of great value, if control tests are conducted using antisera of un-infected persons. We have attempted to confirm the suspected cases of amebiasis by using Gell-immunodiffusion tests and found it useful.

Material and Method

Blood samples of 39 persons were collected to perform immunodiffusion tests. Out of these 28 were patients admitted to Civil Hospital for the treatment of intestinal infection, amongst these 8 had trophozoites of *Entamoeba histolytica* in the stool; others were admitted for various abdominal symptoms.

School of Parasitology, University of Karachi, Karachi.

a) *Serum Preparation*:

About 2-5 ml of blood was taken from each of the patients. Blood was kept at room temperature for 2-6 hours to allow clot formation. The clot was then separated and the serum was centrifuged for about 5-8 minutes at the speed of 1000 r p m to remove any blood cells left in it. It was then stored by freezing as suggested by Morton and Dodge (1963) to avoid deterioration of human precipitins.

b) *Media Preparation*:

The primary purpose of media within which Gell-diffusion tests are performed is to prevent convection currents and to entrap antigen-antibody complex (Crowle 1973). We have used Noble Agar as it is widely obtainable, inexpensive and easy to use. A slight modification of Crowle (1958) microdiffusion test is used to prepare the media, this is done by incorporating 1.5 grams Noble Agar, 0.58 grams sodium barbital, 0.5 grams of Nacl, 1.68 ml of Hcl with 0.1 normality in 100 ml of distilled water.

c) *Casting the Gell*:

The method used is very simple. Two glass slides slightly unequal in size were taken and one of these was coated with 0.2% Noble agar.

At each corner in between the slides was placed a small spacer to get a smooth surface and to get a uniform thickness of the Gell. The smaller slide was kept on the top and hot agar solution (0.5%) was poured between the two slides. In about 10 minutes the agar was solidified and the top slide was gently removed. A very uniform slab of agar was formed by this procedure with a depth of 0.4 cms, holes were bored in it having a distance between central and peripheral holes of 0.45 cms.

The amebic antigen prepared from DKB strain, (from McGill University) was used (Diamond 1968). The antigen was placed in the central well and antiserum of different patients in the peripheral wells with the help of a dropper. The slide was kept on a moist blotting paper in a petridish at room temperature and results were observed after 24 hours.

If a precipitin band appears between the central and peripheral well, it means the antiserum of that well is positive for *Entamoeba histolytica* infection.

d) *Washing and Staining of Precipitin Band*:

For the purpose of washing physiological saline (Ph 7.4 to 8.2) was used since some constituents of antiserum such as euglobulins are insoluble in distilled water. If washing is prolonged additives such as chloroform or sodium azide was added. The washing prior to staining was for about 30 minutes.

In order to make the precipitin bands more intensified staining was done with coomasie blue (0.2%) and then photographed by dark field illumination method as described by Hirschfeld (1960) and Jones and Marshall (1960).

Results

In a total number of 39 patients, since only 8 had living trophozoites of *Entamoeba histolytica* in their stool, it was difficult to diagnose whether all had *E. histolytica* infestation or something else was giving abdominal symptoms. For this purpose Gell-diffusion test was used which confirmed that only 24 out of 39 patients suffering from dysentery had amebiasis. A clear precipitin band was formed in all the 24 cases which suggested that these patients had amebiasis. One of that precipitin band is shown in the following figure.

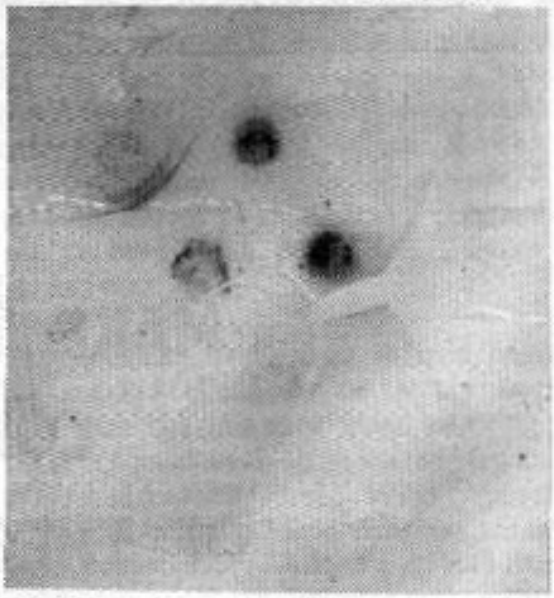

Fig. shows a clear precipitin band stained with coomasie blue.

Discussion

The data presented by early workers such as Juniper et al. (1971) showed that serological test was very useful when skilled parasitological laboratory service was not available and in extraintestinal amebic disease.

Different serological tests for amebiasis were discussed and Gell-diffusion test was found to be the best (Burney and Chaudhry,

1972). They also considered that serological tests should be complementary to stool examination and aspiration of pus from abscess to establish the diagnosis of amebiasis.

E. histolytica infection is very common in tropical and subtropical countries, but in order to properly diagnose the disease very little attention has been paid except on the routine stool test. In most cases the clinical picture of amebiasis is very confusing. A patient may have constipation, either recurrent or chronic, which is usually more common than diarrhoea while other symptoms as noted in patients may include restlessness, fatigability, dull headache, mild fever, nervousness, irritability, sleepiness during the day, joint and muscles pains, indigestion and other obscure digestive disturbances. When stool is examined amebae are not always present. Therefore, serological diagnosis is helpful for the confirmation of amebiasis in suspected cases. This method being easy to perform is of great value in the diagnosis of amebiasis (Dawes 1968).

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References

Burney, A. and Chaudhry, K.N. (1972) Serological tests in amoebiasis. JPMA, 22:341.

Crowle, A.J. Immunodiffusion, 2nd ed. New York, Academic Press, 1973, p. 545.

Crowle, A.J. (1958) A simplified micro double-diffusion agar precipitin technique. J. Lab. Clin. Med., 52:784.

Dawes, B. Advances in parasitology. New York, Academic Press 1968, p. 416.

Diamond, L.S. (1968) Techniques of axenic cultivation of entamoeba histolytica from small inocula. Indian J. Med. Res., 64:572.

Hirschfeld, J. (1960) Characterization of precipitating components in normal human sera obtained by an immunoelectrophoretic technique. Acta Pathol. Microbiol. Scand., 49:255.

Jones, J.H. and Marshall, R.J. (1960) A simple method of photographing Ouchterlony plates. J. Clin. Path., 13:532.

Juniper, K., Worrell, C.L., Minshew, C.M., Roth, L.S., Cypret, T.H. and Lloyd, R.E. (1971) Serologic diagnosis of amebiasis. Am. J. Trop. Med. Hyg., 21:157.

Morton, W. and Dodge, H.J. (1963) Modifications of the agar double diffusion precipitin test for tuberculosis. Amer. Rev. Resp. Ds., 88:264.