Enzyme Linked Immunosorbent Assay (ELISA) in Gut Tuberculosis

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Abstract

A definitive diagnosis of gut tuberculosis often requires invasive tests. The sensitivity and specificity of enzyme linked immunosorbent assay (ELISA) for the diagnosis of tuberculosis have been evaluated in 74 cases with gut tuberculosis, 25 with pulmonary tuberculosis, 25 healthy controls and 25 non-tubercular intestinal disease. These cases were studied for Mantoux test (using 1 T U RT 23) and serum anti PPD titre by ELISA. Mean titre in gut and pulmonary tuberculosis respectively were 2 and 3 times higher than in control groups. The ELISA sensitivity in gut tuberculosis was 76% and in pulmonary tuberculosis 88%. It had a false positivity of 4% in healthy controls and 12% in non-tubercular intestinal disease. Although Mantoux test also had comparable positivity in tuberculosis (77% and 83%), it lacked diagnostic significance due to an almost similar positivity in control groups (63% and 55%).

Thus, ELISA appears to be useful in confirming a diagnosis of tubercular disease.

Keywords: Gut tuberculosis, ELISA, anti PPD titre.

Introduction

Gut tuberculosis is a common problem in our country. Due to its protean manifestations it often presents diagnostic difficulties and often a definitive diagnosis requires laparotomy. The utility of serological techniques in tuberculosis remains unsatisfactory. Although tuberculin test is usually employed for immunodiagnosis, it lacks diagnostic usefulness.

Enzyme linked immunosorbent assay (ELISA), a recently introduced third generation test, has established its usefulness in the serological evaluation of various infections. Its utility in the diagnosis of gut tuberculosis needs evaluation particularly after preliminary reports suggesting promising results in pulmonary tuberculosis.

Material and Methods

Seventy four patients with gut tuberculosis, 25 with pulmonary tuberculosis (having suggestive clinicopathological signs, with sputum for AFB positive in 14), 25 with non-tubercular intestinal diseases (carcinoma caecum/ascending colon—5, intestinal lymphoma—4, appendicitis—4, giardiasis—5, ascariasis—5, non specific lymphoid hyperplasia—2), and 25 matched healthy controls with normal chest radiographs were studied. Patients with gut tuberculosis were selected from 88 clinicoradiologically diagnosed cases, who had diagnostic operative findings (laparoscopy, minilaparotomy/laparotomy) along with either confirmatory histology (55) or subsequent positive therapeutic response (19).

Mantoux test (first strength) was performed by injecting 0.1 ml of PPD RT 23 containing 1 T U intradermally on the flexor aspect of the forearm. The response was read after 72 hours and induration of 10 mm or more in the long axis of the forearm was taken as positive.

ELISA: Sera were collected from various groups of cases (before starting specific therapy in tubercular cases) and stored at —20°C. ELISA for estimation of serum anti PPD antibodies was standardised and carried out based on techniques described earlier. Antigen used was purified tuberculin (PPD) obtained from Statens Serum Institute, Denmark and the microtitre ELISA plates from Dynatech Laboratories, Alexandria, USA. The enzyme conjugate used was horse radish peroxidase conjugated rabbit anti-human polyclonal immunoglobulins obtained from Dakopatts, Denmark (no Freund's adjuvant is used during production of these antisera in order to eliminate the chances of contamination with antimiobacterial antibodies). Serum was tested in single dilution (1:100) in duplicate wells. Optimal dilutions obtained by checker board titrations were, for PPD, 10 μg/ml, serum 1:100 and conjugate 1:1000 (in PBS pH 7.2). 0-1 ml of these were used with 0-2 ml of 0-phenylene dimine dilydrochloride as enzyme substrate. The reaction was allowed to proceed in the dark for 30 min, then stopped with 0-05 ml of 5N H2SO4. Optical density (OD) was recorded by spectrophotometer (Beckman DU-6) at 492 nm and titer were expressed as OD x 100. If duplicate wells gave readings varying by more than 0-005, the test was repeated.

ELISA titres greater than mean +2 SD of healthy controls were considered positive. 2 X 2 table and chi square test were used to obtain significance of ELISA, as well as Mantoux positivity. Student's t test was used for significance of mean titres in different groups. Positive and negative predictive value and efficiency of the test were calculated as described by Briggs.

Results

A majority (66.2%) of patients with gut tuberculosis were in the 2nd and 3rd decades of life, with females outnumbering males 1:5:1. Among the 33 cases with intestinal obstruction, females predominated by about 5:1. Predominantly acetic presentation was seen in 16 subjects. Associated pulmonary tuberculosis was
observed in 31% and cervical/axillary lymph node involvement in 9-5%. Although endometrial biopsies were not studied for genital involvement, about 31% of females had amenorrhoia/oilomonoamenorrhea.

Mean ELISA titres for anti PPD antibodies in gut tuberculosis (38 ± 12) and pulmonary tuberculosis (51 ± 11) were about 3 folds and 3.5 folds higher respectively (p<0.001) as compared to healthy (16 ± 5) and non tubercular disease (17 ± 7) controls. ELISA was positive among 76% of patients with gut tuberculosis and 88% with pulmonary tuberculosis compared to only 4% positivity among healthy subjects (Χ² = 36.8 & 35.4 respectively; p<0.001) and 12% among nontubercular disease controls (Χ² = 32.1 & 28.8 respectively; p<0.001). Mantoux test positivity in cases with pulmonary (83%) and gut (77%) tuberculosis was statistically not different from that in healthy controls (63%). Non tubercular intestinal disease controls also had high (55%) Mantoux positivity, but compared to the tubercular disease group it was lower (p<0.05).

Predictive values of ELISA positive and negative were 95% and 69% respectively with efficiency of 82%, whereas predictive values of Mantoux positive and negative were 74% and 48% respectively with efficiency of 66%. When the specificity of the test was raised to 100% (considering values greater than the highest among both the control group as positive), ELISA remained positive (titre > 31) in 69% of cases with gut and 84% with pulmonary tuberculosis whereas Mantoux positivity (> 15 mm) dropped to 25% and 52% respectively.

The different clinical presentations of gut tuberculosis, viz obstructive, non-obstructive and predominantly ascitic cases, had statistically similar mean ELISA values (39 ± 14, 36 ± 11 and 39 ± 13 respectively) and similar ELISA positivity (79%, 68% and 82% respectively). Mean ELISA titres and ELISA positivity in Mantoux positive and negative controls were statistically similar, but all the ELISA positive controls were Mantoux positive as well. Although Mantoux positive gut tuberculosis cases had higher ELISA titres than negative cases (p<0.001), the titres were similar in pulmonary tuberculosis patients regardless of Mantoux positivity (Table).

Discussion

An invasive approach is not always feasible nor is it acceptable in the day to day diagnosis of gut tuberculosis. One has to therefore rely usually on clinico-radiological evidence, which may often be fallacious.1,4,14

The high sensitivity of ELISA in gut tuberculosis (76%) and pulmonary tuberculosis (88%) with a high specificity of 92% as against similar yield with histology (72%), speaks of its usefulness in detecting tuberculosis. Moreover, ELISA positivity and antibody titre were similar irrespective of histology being positive or not.

ELISA also had high predictive value (reliability) of positive (95%) and negative (69%) results, with an efficiency of 82%. This high true predictive value (diagnosing a specific disease when it exists) is not only related to high sensitivity and specificity of the test but also to high prevalence of disease in subjects studied (99/149 or 66%), as highlighted by Braganza.17 However, this is in accordance with the clinical situation, because the test is intended to be used for confirming diagnosis in clinico-radiologically suspected cases, where the prevalence of the disease is likely to be high (74/88 i.e. 84% in this study).

Although the sensitivity of Mantoux test was comparable to ELISA, its specificity of 41% was much poorer than the value of 92% for ELISA (Χ² = 27.5; p<0.001). When the tests were interpreted with 100% specificity, ELISA positivity was only marginally altered, whereas the value of Mantoux positivity was considerably reduced, particularly in gut tuberculosis cases (25%). Moreover, earlier studies reported highly variable Mantoux positivity, ranging from 30%10 to 85%11 in gut tuberculosis. There are no reports on ELISA in gut tuberculosis, but the reported positivity of 80-84% with only 4-8% false positivity in pulmonary tuberculosis12,13 suggests its usefulness. In other studies, ELISA test could discriminate patients with pulmonary tuberculosis from control subjects, but its usefulness was limited due to overlapping titres in many cases and day to day variation of titre.14,15 Benaim and Daniel,16 in contrast to these findings and in conformity to our observations, found high specificity of ELISA in the diagnosis of pulmonary tuberculosis. Only limited (11%) positivity in Mantoux positive healthy and non-tubercular controls in this study suggests that anti PPD titres reach diagnostic levels only if active tuberculosis exists.

The relationship of antibody type and titre with active and healed tuberculosis was not evaluated in this study. Though IgM antibody response is anticipated in active disease, available reports reveal it to be of not much diagnostic utility.13 Hence follow up ELISA studies in patients receiving anti-tubercular treatment need to be undertaken to ascertain the exact significance of raised titres. On the present evidence, ELISA appears to be of value in detecting tuberculosis, and could be of help particularly in gut tuberculosis with non-obstructive and ascitic presentations, where surgery is not indicated.
References

With Best Compliments from

CHEMIST ASSOCIATION

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