Original Article

Immunochromatograhic TB Test in Tuberculosis

Diagnosis of Active Tuberculosis with Immunochromatograhic TB Test in Suspected Tuberculosis Patients

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ABSTRACT

Objective: To detect active disease in suspected cases of pulmonary and extrapulmonary TB patients we employed immunochromatographic IgG/IgM antibodies using recombinant antigen kDa 6, kDa16 and kDa 38, which has higher sensitivity and specificity.

Study Design: Prospective cohort study

Place and Duration of Study: This study was conducted at the Department of Medicine in collaboration with Radiology and Pathology Departments of Pak Red Crescent Medical and Dental College Teaching Hospital Dina Nath from March 2016 to March 2018.

Materials and Methods: A sample of Fifty four suspected TB patients with the same number of control patients were included in this study. The patients were divided in different groups as (1) 45 patients with clinical features of suspected pulmonary tuberculosis, meeting WHO criteria, (24 new and 18 old) and 3 cases of pleural tuberculosis, along with chest X-ray (CXR) had findings with suspicion of tuberculosis.(2) Nine patients with diagnosis of suspected TB lymphadenitis.(3) Forty five non-suspected TB patients with asthma, chronic obstructive pulmonary disease (COPD) and others with minor respiratory tract complaints, without constitutional symptoms but mimicking tuberculosis and having no evidence of TB findings on chest films were taken as controls. Similarly 9 controls with lymph nodes enlargement but without any suspicion of tuberculosis were taken as controls. The chest X-rays were taken in Radiology department and the laboratory investigations and ICT-TB test were performed in the pathology department of hospital.

Results: Sensitivity in newly symptomatic patients of pulmonary and extrapulmonary disease with rapid TB tests was 66% while in old healed TB cases was 16% with 100% specificity and high positive predictive values. Our focus was on finding and results in suspected cases of tuberculosis with the help of clinical features ,chest x.ray findings ,and confirming these with ICT-TB test.

Conclusion: Rapid TB test is helpful in early and rapid diagnosis of tuberculosis in suspected patients.

Key Words: Pulmonary and extra pulmonary tuberculosis, IgG, IgM, Chest X-ray, Recombinant antigen

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INTRODUCTION

Tuberculosis is prevalent in one third of the population of world and occurs in poor socioeconomic conditions and in immune compromised patients.[1,2] It is active only in ten percent of cases.[1] In Pakistan active tuberculosis is prevalent in nearly 518000 patients as per WHO reports.[3] TB is totally curable disease but still a major killer in infectious diseases.[4] In pulmonary TB the disease spreads by coughing and sneezing. It usually is diagnosed by conventional methods of sputum smear positivity test which has poor specificity and prolonged culture results although with high sensitivity.[5,6] Chest X-rays findings in newly diagnosed cases are usually nonspecific.[7,8]

Non availability of sputum sample is another problem in extra pulmonary TB which is prevalent in upto 15-20 percent of tuberculosis cases in Pakistan and rest of the world[9,10] with major presentation with lymphadenopathy followed by pleural effusion and other extra pulmonary foci.[11,12]

The immune mediated tests such as manteaux test, gamma interferon tests as quantiferon-TB gold, spot TB tests with variable sensitivities but cannot differentiate between active and latent tuberculosis.[13-15] So serodiagnostic tests against specific antigens have variable sensitivities but good specificities in detection of active tuberculosis.[16-19] Rapid ICT test for detection of serum immunoglobulin's IgG/IgM raised against 38kDa 16kDa, 6kDa antigens showed good results for rapid diagnosis of active tuberculosis , with smear positivity in national and international studies with 100 percent specificity and good sensitivity.[20,21] In the

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present study we tried to detect active TB by ICT tuberculosis test with upto 100 percent specificity in the patients suspected to be suffering from TB with positive clinical feature of this disease and/or relevant X-ray findings in pulmonary and extrapulmonary cases.

MATERIALS AND METHODS

A prospective study with the approval of the ethical committee of the college was conducted. The patients enrolled in the study were adults 54 in number males& females from the nearby population who visited hospital in last 2 years from March 2016 to March 2018 with or without previous history of tuberculosis who meet the criteria as following.

For tuberculosis suspected patients; These forty five patients (24 fresh, 18 with previous h/o TB and three with pleural effusion in whom Tuberculosis was suspected) with either history of cough, fever & weight loss for more than 2 weeks or cough with less than 2weeks of uncertain origin plus either bloodstained sputum , fever at night and/ or weight loss in fresh or old and treated TB patients.[5] The patients CXR were taken & only those with radiological findings of infiltration, cavitation or consolidation in 24 fresh cases and /or with findings suggestive of old tuberculosis in 18 cases that is opacities, fibrosis and mediastinal shift were included in study for pulmonary TB[7,8], and 3 patients with suspicion of TB with chest X-ray findings of pleural effusion. (3) The nine patients with cervical, mediastinal lymphadenopathy with symptoms of fever, loss of weight and anorexia. The cervical lymhadenitis cases have either matted or in discharging stage.

For Control patients: Forty five patients with minor respiratory problems or asthma and chronic obstructive pulmonary disease without any constitutional symptoms of tuberculosis and with normal chest X-ray were taken as controls in pulmonary cases, 2 for controls in lymphadenitis cases. The patients were selected with isolated cervical lymphadenopathy of short duration and without any constitutional symptoms of tuberculosis.

Laboratory investigations for TBIgG/IgM immune chromatographic test is based on detection of IgG and IgM raised against one or more of the three 16kDa, 6kDa, 16kDa recombinant antigens. The device has letters C (control line) M (TB IgM) test line and G (TB IgG) line. The devise used is manufactured by Healgen Scientific LLC:USA . It is used in the following steps. (1) Take fresh serum /plasma from blood as soon as possible .Stored serum may be used if kept in refrigerator at 2-8°C but should be brought at room temperature at the time of use. (2) Briefly 10 microliter of serum was added to square sample well and then 4 drops assay diluents provided in the kit was added. Interpretation of test took 15 minutes; ICT does not require special skill and equipment and (3) the results can be interpreted according to change of color of band G or M. C band must however be positive, otherwise results are wrong.

Principle underlying the ICT TB kit: The test uses a nitrocellulose membrane strip containing two lateral bands (IgG & IgM) Bands and a control band C. The M band is pre-coated with anti-human IgM antibody. The G band is pre-coated with anti-human IgG antibody and the C band is pre-coated with anti-goat antibody. The burgundy colored conjugate pad contains colloidal gold conjugated to recombinant TB specific antigens kDa38, kDa6, kDa 16. When a specimen followed by assay buffer is added to sample well IgM &/or IgG antibodies if present will bind to TB conjugates making antigen antibodies complex which will traverse through nitrocellulose membrane by capillary action. When the complex meets the corresponding immobilized antibodies (anti human IgM and /or anti human IgG) the complex is trapped forming burgundy coloured band which confirm a reactive last result and vice versa. The test contains an internal control (c band) which should exhibit a burgundy colored band of the anti-goat antibody; otherwise the test result is invalid. The data was entered and analyzed by SPSS-20.

RESULTS

Fifty four (male and female) patients in different categories as fresh pulmonary 24 in number(13 males and 11 females) lymphadenopathy cases 9 in number (2 males and 7 females), three pleural effusion (2 males and 1 female) 18 old cases (10 males and 8 females) with suspicious of relapse in age ranges shown against each group (Table 1).

Total pulmonary (fresh and extra pulmonary 24 and old 3/18) 27 positive TB cases were found. Out of these only 4 were found IgM positive (3/36 fresh positive TB cases and extra-pulmonary) and 1/18(previously treated pulmonary tuberculosis patients), accounting for 14% total IgM positive patients). 21/36 (58 %) patients in

Table No.1: Demographic data of selected patients

Variable	No.	Male/Female	Mean age (Male/Female)	Age range (Male/Female)
Fresh cases of Pulmonary tuberculosis	24	13/11	44/35	18-60/14-100
Lymphadenopathy	09	02/07	22/21	20-30/05-33
Tuberculosis with pleural effusion	03	02/01	30/28	30/25-28
Old TB with relapse (symptomatic)	18	10/08	42/38	18-75/8-90
Total	54	27/27	36/29	18-75/8-90

Table No.2: Number of positive cases for IgM/IgG antibodies in rapid TB ICT in suspected cases

Type	Total suspected cases	Positive TB cases	TB IgM +	TB IgG +
New positive cases of respiratory tract infection + extra pulmonary tuberculosis	36	24	3 (08%)	21 (58%)
Pulmonary tuberculosis	24	13	-	13 (54%)
Extrapulmonary tuberculosis (lymph node and pleural)	12	11	3 (08%)	8 (66%)
Previously treated TB cases with symptoms	18	3	1 (5.5%)	2 (11%)

Table No.3: Total number of rapid IgM/IgG antibodies test for TB recombinant antigen positive cases with percentages sensitivity specificity positive predictive negative predictive values and confidence interval

Type	Total IgM & IgG	Sensitivity (C.I) 95%	Specificity	PPV	NPV
New cases of pulmonary tuberculosis 36	24	66% (0.56-0.77)	100%	100%	75%
Pulmonary tuberculosis 24	13	56%	100%	100%	67%
Extrapulmonay tuberculosis	11	84%	100%	100%	88%
Old cases with relapse 18	3	16% (0.1-0.17) 100%	100%	100%	54%
Total cases 54	27	50%	100%	100%	50%

fresh cases whereas 2/18 (11%) previously treated tuberculosis cases were found to be IgG positive (Table 2).Total new cases with IgM & IgG collectively show 66% sensitivity with 95% confidence interval of 0.56 to 0.77 as compared to those with previous H/O treated TB cases of 16% with confidence interval of 0.1-0.17 (Table 3).

DISCUSSION

The various tuberculosis bacilli components, consisting lipid, protein antigens have been employed in different serodignostic tests for active TB detection with different results.[16-19] The non-specific Manteaux tests and Interferon gamma releasing assay tests cannot differentiate the active from inactive diseases.[9-11] The different serodiagnostic tests with various purified protein antigens alone as kDa 38 antigens[16] or Lipoarabinomannan (LAM) antigens a lipid antigen[14], or in combination as pathozyme myc (LAM +38kDa) with 21-46% sensitivity and pathozyme complex TB (38 kDa+16kDa) with sensitivity of 51% were employed in various studies with a specificity of 94-100%.[13-15] However in a study by Selma et al[21] reported that IgG/IgM mediated antibody responses against mycobacterial 38kDa, 16kDa, 6kDa6 in active tuberculosis with positive sputum smear in pulmonary and extrapulmonary tuberculosis IgG and IgM were 68.4% and 2.3% respectively.[19] Similiarly in a study for detection of TB by Khan et.al[20] in Pakistan, performed on 129 cases antibody was found positive in 23/52 sputum positive 11/36 in sputum negatives 10/30 of pleural effusion and 6/16 of TB lymphadenitis case. Specificity was 100%.

The results of our present study shows the positive results with rapid IgG/IgM antibody test for TB recombinant antigens 38kDa, 16kDa, 6kDa in fresh tuberculosis suspected cases as 66% and upto 16% in healed old tuberculosis cases. So the sensitivity of these results showed 66% and 4% in fresh and old healed case respectively. As the specificity of this test is found as 100% in our study as is claimed in other studies in sputum smear positive cases.[20,21] So our study in suspected cases of tuberculosis in detection of active tuberculosis is very encouraging as we may find the results in a large number of cases without indulging in smear positivity and long awaiting culture positive results. In our study IgM response is very poor i.e. 16% as compared to IgG response which is 66%. As it is known IgM is positive in early stages of disease, while IgG response comes later. The discrepancy found in our study may be due to quackery and deficiency of qualified doctors, so by the time the patient contacts a qualified doctor, it is already very late and we get poor IgM positivity in our cases. Another finding in our study is that the patients selected with the suspicion of relapse in old treated cases showed positivity in a very few cases (16%) which may be due to the fact that these patients have developed complications such as bronchiectasis, pulmonary abscess, and repeated nonspecific respiratory tract infections, because of previous tuberculosis, So most of the time it is difficult to diagnose tuberculosis with relapse cases clinically. However in positive cases we may declare that the patient is in relapse.

Also there are other lipid antigens such as cord factor TDM (trehalose 6, 6 dimycolate) a major part of mycobacterial cell wall is the most immunogenic

glycolipid in ELISA reported to have a sensitivity 81% and specificity of 96%[22] and maybe employed in our population for early detection of tuberculosis.

CONCLUSION

The rapid test for IgM/IgG antibodies against recombinant antigen kDa38, kDa16 and kDa6 is very helpful in detection of suspected tuberculosis cases which has higher sensitivity and nearly 100% specificity. This may be used as primary test for detection of active disease. Also in sputum smear negative cases the test may be helpful in detection of disease earlier, as conventional culture test may take 4-6 weeks for results. Because of poor sensitivities of the test the patients with negative ICT TB test has limitations in suspected cases and they must be further evaluated with culture and sensitivity tests.

Author's Contribution:

Concept & Design of Study:	Arif Gulzar
Drafting:	Nasir Mahmood
Data Analysis:	Fizza Muazzam
Revisiting Critically:	Arif Gulzar, Nasir Mahmood
Final Approval of version:	Arif Gulzar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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