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Research Article

Qualitative analysis of IgM antibody in tuberculosis

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ABSTRACT

Background & Objectives: Nearly one third of the global population is infected with *mycobacterium* tuberculosis and at risk of developing the disease. More than eight million people develop active tuberculosis every year and about two million dies. Thus, this study was evaluated to study of the sensitivity and specificity of IgM antibody by ICT (Immuno-chromatographic test) method in diagnosing tuberculosis.

Materials and Methods: This study comprised of 100 subjects, of which 25 were sputum positive tuberculosis, 25 were sputum negative tuberculosis diagnosed on the basis of clinical and radiological backgrounds, and 50 subjects were taken as control of which 25 were chest infections other than tuberculosis and the rest of 25 were clinically normal subjects.

Results: The sensitivity and specificity of IgM antibody was found to be 32% and 94% in the AFB positive group, where as in the AFB negative group it came out to be 24% and 94%, overall, on combining both the group the sensitivity and specificity of IgM antibody came out to be 28% and 94%.

Conclusion: Serum IgM antibody test has a low sensibility in diagnosing tuberculosis, but it can be helpful in excluding tuberculosis in individuals suspected to have tuberculosis.

Keywords: IGM antibody, *Mycobacterium* tuberculosis, Sensitivity, specificity

INTRODUCTION

Mycobacterium causes disease of enormous importance in both humans and animals. Tuberculosis, a specific communicable disease, is a mycobacterial disease of human beings. It is an ancient disease of man and still one of the widest spread diseases despite

modern methods of effective control. Nearly one third of the global population, i.e. more than two billion people is infected with Myocabacterium tuberculosis and at risk of developing the disease. More than eight million people develop active tuberculosis every year and about two million dies. More than 90% of global TB cases and deaths occur in the developing world, where 75% of the cases are in the most economically productive age group (15-54 years). This results in the loss of 20 - 30% of annual household income and if the patients die of TB, an average of 15 years of lost income. In addition to the devastating cost TB imposes indirect negative consequences. Children leave school because of their parent's tuberculosis and women are abandoned by their families as a result of their disease [1]. Indeed, the percentage of negative result has been estimated to be between 30 & 50% in developed countries. At the same time, it cannot be applied in children because they rarely produce sputum [2,3]. Sputum culture is time consuming.

Although often the basis of suspicion, X-ray findings have been estimated as atypical in more than 30% of patients with tuberculosis in developed countries [4]. The TB IgM Rapid Test is a one-step chromatographic immunoassay, which specifically detect the antibodies to Mycobacterium Tuberculosis in human serum or plasma. During the test, TB antibodies (IgM) if present in the patient sample migrate through the conjugate and where they bind to the conjugates. The antibody conjugates are then captured by anti human IgM immobilized on TI region, forming a burgundy-colored band on the test region (TI), indicating a positive test result. Thus, this study was evaluated to study of the sensitivity and specificity of IgM antibody by ICT (Immuno-chromatographic test) method in diagnosing Tuberculosis.

MATERIALS AND METHODS

This study comprised of 100 subject presented in the department of Internal Medicine, Janaki Medical College Teaching Hospital, Janakpur, Nepal during the period of 02.01.2078 BS (15.04.2021 AD) to 30.12.2078 BS (13.04.2022 AD), of which 25 were sputum positive tuberculosis, 25 were sputum negative tuberculosis diagnosed on the basis of clinical and radiological backgrounds, and 50 subjects were taken as control of which 25 were chest infections other than tuberculosis and the rest of 25 were clinically normal subjects.

Clinical evaluation of Cases

Sera from 50 patients were collected, who were indoor patient, admitted in the department of Internal Medicine of Janaki Medical College Teaching Hospital, Janakpur. None of the either sputum positive or negative group was on anti-tuberculosis treatment (ATT) at the time of blood collection. Thorough clinical evaluation of all these patients were done on the basis of the proforma designed for that purpose. A detailed history was taken with particular emphasis on cough, fever, chest, pain, hemoptysis, loss of appetite, loss of weight and breathlessness. The relevant data pertaining to personal history, past history, family history socio-economic history and history of tuberculosis in past were recorded. Chief complaints of the patients were recorded in chronological order with duration. All the chief complaints were elaborated by asking leading questions. Then general and systemic examinations for each patient were done carefully. In systemic examination, chest was examined under the

headings of inspection, palpation, percussion and auscultation, positive and important negative findings were noted.

Control

Sera from 50 control subjects were taken, control included the chest infection group who were diagnosed on the basis of clinical and radiological findings and the other group included of clinically normal subjects with no past or family history of tuberculosis. All the subjects were above sixteen years of age.

Investigation

- Routine investigations
- Specific tests
    - Sputum Examination
    - Mantoux Test (Standard Tuberculin Test)
    - Radiographic study
    - Sero-diagnosis for Tuberculosis

Assay of serum IgM

Collection of samples: 5 ml of blood samples was collected from each patient and from individuals from the healthy group using normal aseptic technique with 5 ml. disposable syringe and 20 gauze needles in sterile vials. Blood sample were allowed to clot at room temperature for two hours and then the samples were centrifuged at 2000-3000 rpm for 10 minutes, supernatant was transferred into capped sterile tubes. Hemolyzed samples and samples showing a strong lipemia or turbidity were discarded.

Performance of assay:

The test was performed by CTK biotech TB Antibody IgM onsite rapid Kit, the TB IgM Rapid Test. (photograph No-1) this kit was provided with the following components:

Each kit contains 25 test devices, each sealed in foil pouch with three items inside.

a) One cassette test device.

b) One pipette dropper.

c) One desiccant

Test procedure

- Test device, buffer, and serum or plasma specimen, were allowed to equilibrate to room temperature (15-30^{0}c). Prior to testing was done.
- When ready to test, the pouch at the notch was opened and the test device was removed, and was placed on a clean flat surface.
- The device was labeled with specimens ID.
- The pipette dropper was filled with the specimen and holding the dropper vertically, 2-3 drops (about 50-90 ul) were dispensed into the sample well without air bubble, and the timer was set up. One drop (about 30 ul) of saline of PBS balance buffer was added into the sample well if flow migration was not observed in 30 seconds in the result window, which could have occurred with high viscous specimen.
- The test result was read in five (5) to ten (10) minutes after adding the specimen. Positive result was visible as short as 1 minute.
- Result was noted after 10 minutes.

Interpretation of result

Negative Result

If only the C line is present, the absence of a line in region (TI) indicates that no TB antibodies are detected. The result is negative.

Positive Result

In addition to the presence of C line if T1 line is developed it indicates presence of IgM antibodies to M.TB.

Invalid Result

If no C line develops despite of the presence of T1 line the test is considered to be invalid.

In this study 50 patients and 50 controls were included. As already describe in the previous section a thorough evaluation of the patients was done clinically, radiologically and bacteriologically.

The patients group comprised of 38 males and 12 females, in control group also, there were 38 males and 12 females. Mantoux test was considered positive of a diameter of more than 10mm. Criteria for the diagnosis of tuberculosis in patients were history, clinical examination, radiological examination, blood test, mantoux text and sputum examination.

Majority of the patients (80%) of this study group were having symptoms of less than 2 months duration, and the rest 20% had 2 to 4 months old symptoms. Among patients, that is sputum positive and the negative group 27.4% patients gave history of contact of tuberculosis either in the family or in the neighbors, in last 2-3 years. Majority of the patients (76.7%) of this were belonging to poor socioeconomic class. In this study only those patients were included who were not on antituberculosis treatment at the time of admission.

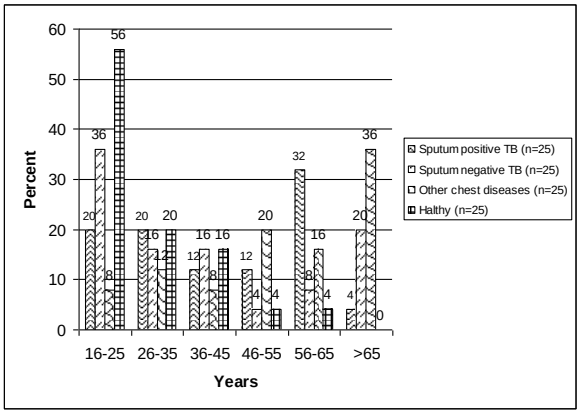

Fig 1: Age distribution in four groups (n=100)

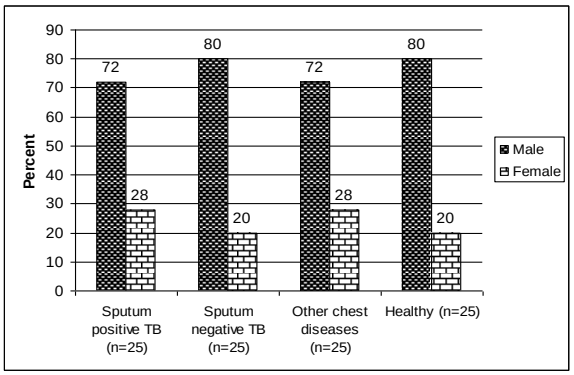

Fig:2 Sex distribution in four groups (n=100)

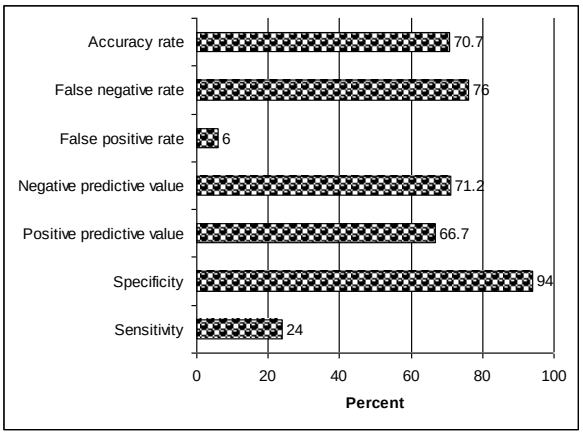

Fig 3: Predicting TB (sputum negative) by IgM

RESULTS

In this study four groups were divided: i) Sputum positive tuberculosis group, ii) sputum negative tuberculosis group diagnosed on the basis of clinical and radiological findings, iii) chest infection other than tuberculosis group which consisted of pneumonia, COPD, and iv) the healthy individual group. In most cases cough was productive. Table 1 depicts that all patients with pulmonary tuberculosis had productive cough. Loss of appetite was present in 88% in sputum positive group and 84% in the negative group. Loss of weight was found in 80% in the sputum positive group and 60% in the negative group. Hemoptysis was present in 84% in the sputum positive group and 40% in the negative group. IgM antibody test for tuberculosis was done in each group and the

results were compared. In the first group that is the sputum positive group IgM antibody was positive in 32%, In the negative group it was positive in 24%, in the chest infection group it was positive in 8% where as it came out to be positive also in 4% of the healthy individual group.

Fig 2 explains the disease prevalence was found more in males, 72% were sputum positive and 80% were sputum negative while in females 28% were sputum positive and 20% were sputum negative (p-values 0.831).

Fig 3 depicts that then comparing the result of the sputum negative tuberculosis group with the control group which included the healthy and the chest infection group the sensitivity and the specificity of IgM antibody test came out to be 24% and 94% respectively (p value 0.053).

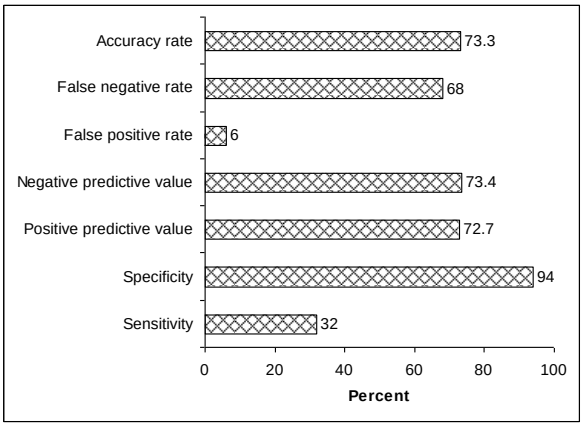

Fig 4: Predicting TB (sputum positive) by IgM

Table 1: Distribution of the patients according to complains

SOB and cough	**Group**			**p-value**
	Sputum Positive TB	**Sputum Negative TB**	**Other chest disease**	0.010
Present	23(92.0%)	20(80.0%)	14(56.0%)	
Absent	2(8.0%)	5(20.0%)	11(44.0%)	
Total	25(100.0%)	25(100.0%)	25(100.0%)	
Loss of appetite				p-value
	Sputum Positive TB	**Loss of appetite**	**Sputum Positive TB**	< 0.001
No	3(12.0%)	No	3(12.0%)	
Yes	22(88.0%)	Yes	22(88.0%)	
Total	25(100.0%)	Total	25(100.0%)	
Loss of weight	**Group**			p-value
	Sputum Positive TB		**Sputum Positive TB**	0.001
No	5(20.0%)	No	5(20.0%)	
Yes	20(80.0%)	Yes	20(80.0%)	
Total	25(100.0%)	Total	25(100.0%)	
Hemoptysis	**Group**			p-value
	Sputum Positive TB	**Sputum Negative TB**	**Other chest disease**	0.0899
Present	21(84.0%)	10(40.0%)	3(12.0%)	
Absent	4(16.0%)	15(60.0%)	22(88.0%)	
Total	25(100.0%)	25(100.0%)	25(100.0%)	
IgM antibody for TB	**Group**			p-value
	Sputum Positive TB		**Sputum Positive TB**	0.026
Negative	17(68.0%)	Negative	17(68.0%)	
Positive	8(32.0%)	Positive	8(32.0%)	
IgM antibody for TB	**Group**	**IgM antibody for TB**	**Group**	

Fig. 4 compares the results of the sputum positive tuberculosis group with the control group, the sensitivity and the specificity of IgM came out to be 32% and 94% respectively (p values 0.005).

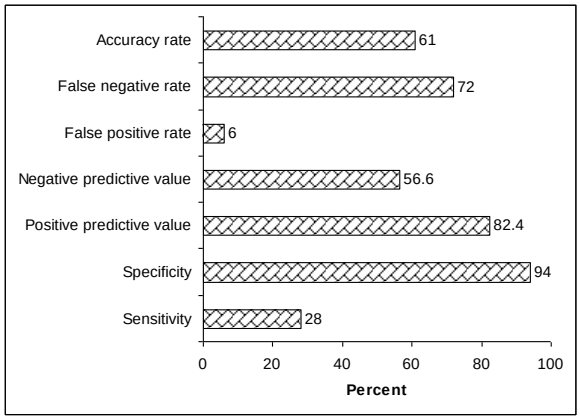

Fig 5: Predicting TB (sputum positive + sputum negative) by IgM

Fig 5 depicted that when the results of sputum positive and negative group was compared as a whole with the control group the sensitivity and specificity of IgM came out to be 28% and 94% (p values 0.008).

DISCUSSION

Despite the large amount of attention currently being focused on AIDS, tuberculosis unassociated with HIV infection still accounts for greater morbidity and mortality. Methods of diagnosis have changed little in past 50 years and most laboratories still rely on Ziehl-Neelsen Microscopy for rapid diagnosis. The search for an ideal serologic test has been on since the end of the 18th century. An agglutination test was described by Airlong, as early as 1898 [5]. Soon the technology gained imperious and various complement fixation tests, hemagglutination test, agglutination of inert particles coated with antibody, precipitation and gel diffusion test, fluorescent antibody test [6] [38] came in vogue and vanished with the passage of time as all these tests in spite of several modifications, faced the problem of overlap between diseased and skin test positive healthy subjects. The antigen profiles of mycobacteria are very complex. They consist of purified protein derivatives, protein A, B and C, carbiomannan and glycolipids like wax D, phosphotidyl inositol, monoxides, sulphatides and myocosides [7]. Many of these antigens are shared by various mycobacterial species and to a lesser extent by other genera. This has been one of the important factors contributing to the non-specificity of the immunologic detection system. The advent of purified and recombinant antigens, monoclonal antibodies and gel separation technique has done much to overcome this with specific mycobacterial antigens and improved immunologic techniques the proportion of false positive in tuberculosis serology testing has been reduced significantly.

Smear positive patients can be diagnosed bacteriologically with ease and certainty, so that it remains unlikely that serology, at the efficiency known, could do much to improve their diagnosis, but the diagnosis of sputum negative tuberculosis is difficult, delayed in many cases and frequently made on circumstantial evidence alone. The clinical manifestations are varied and often mimic those of other diseases. Sero-diagnosis could be of great value in smear negative patients. Keeping the value of sero-diagnosis in mind this study was conducted on 50 patients of tuberculosis. Among those patients 25 were bacteriologically proved cases of tuberculosis and the rest of 25 were diagnosed on the basis of radiological and clinical findings. In this study patients belonged to the 16 to 65 years age range with a maximum prevalence of disease in third to fifth decade of life, (fig

1). Majority of the patients in this study presented with cough for 1 to 3 months of duration as predominant symptom. This finding was in accordance with findings of Banerjee D and Anderson S [8]. Fever, loss of weight, pain chest, loss of appetite and hemoptysis were other chief complaints of the patients. These were found in agreement with WHO chronicle. In this study more than 75% patients were belonging to poor socioeconomic class, this finding was in accordance with reports of Styblo K21 that standard of living and quality of life are important factors in causation of tuberculosis. In this study the sensitivity and specificity of IgM came out to be 28% and 94% which were similar with findings of, Ongut. G.et al [9] where the sensitivity, specificity, and negative predictive value of the Immunochromatographic Test (ICT).

Tuberculosis test for pulmonary TB were 33.3%, 100% and 52.9%, respective. Smear-positive pulmonary TB patients showed a higher positivity rate for antibodies than smear-negative patients, but the difference was not statistically significant.

A study which was conducted at Department of Biochemistry Mahatma Gandhi Institute of Medical Sciences Sevagram, Wardha, and Maharashtra, India analyzed antigen specific immunoglobulin IgM, in clinically and bacteriologically confirmed pulmonary tuberculosis cases to determine the usefulness immunoglobulin in the diagnosis of patients attending the hospital. Of the 30 cases of pulmonary tuberculosis 19 (63.3%) were positive for IgM [10]. However, in this study out of 25 cases of sputum positive pulmonary tuberculosis 8 (32%) were IgM.
In this study the sensitivity of IgM varied from 24 to 32% in the sputum negative and the positive group and combining the both group the sensitivity was 28% which was similar with the finding of a study done study done at Department of Laboratory Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, and 2 Warsaw Medical School, Warsaw, where Sensitivity of IgM came out to be 32% [11]. Ima et al. studied Sera from 58 patients with pulmonary TB, and 45 subjects with other underlying disorders (control group) were analyzed. The sensitivities of the test ranged 29%. The specificity of the test was 93% the finding was accordance with this study [12]. Zielonka TM et al. evaluated serologic response in group of 270 patients. 137 patients with active tuberculosis (TB), 15 patients with mycobacterial infections other than TB, 58 patients with Sarcoidosis, 26 patients with lung cancer and 34 healthy controls. Specificity for IgM of 85%, Sensitivity of 18% for IgM the sensitivity was low as compared to this study [13].

Kalantri Y et al. evaluated sera from 114 healthy volunteers, 105 bacteriologically confirmed cases of pulmonary tuberculosis (PTB), 59 sera from family contacts of PTB, and 40 sera from cases of lung infections other than tuberculosis and found the sensitivity for IgM was low (28.5%) but the specificity was high (95.7%). None of the 40 no tubercular lung infection cases were positive for the IgM, the finding of which was similar to the finding of this study [14].

Mahajan et al. evaluated a sero-diagnostic test in patients with active tuberculosis (Group I), clinically suspected pulmonary tuberculosis (Group II) and pulmonary diseases other than tuberculosis and normal healthy subjects (Group III) and compared with culture and sputum smear examination results. IgM was

found to have a sensitivity of 77.5% and a specificity of 87.5%. In patients clinically suspected to have pulmonary tuberculosis IgM had a sensitivity of 60% the finding of which was not consistent with this study which could be possibly due to the difference in methods used [3]. This study was limited to one tertiary center of Nepal with limited sample size. So, it cannot be related all over the Nepal.

CONCLUSION

The maximum prevalence of disease was in males and cough was the predominant symptom in most of the patients. The sensitivity of IgM came out to be low in both the groups but it as much lowers in the sputum AFB negative group; however, the specificity was equally high in both the group so from this study it can be concluded that serum IgM antibody test has a low sensitivity in diagnosing tuberculosis, but it can be helpful in excluding tuberculosis in individuals suspected to have tuberculosis.

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Author's Contribution: Concept, design, supervision, data collection, literature review, writing of manuscript- **RNM, AKM, ELM, MRM;** reference management, final revision of manuscript. Each author provided their final approval for the intended publication.

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