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Iga Response To 38 Kda And 27 Kda Antigen Of Mycobacterium Tuberculosis In Genitourinary Tuberculosis.

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----------Abstract----------

Objective :*To evaluate the isotype IgA and IgG response in genitourinary tuberculosis (GUTB) to mycobacterial purified cell wall antigens of 38KDa, 16KDa, antigen 85 complex and recombinant antigen 27 KDa by enzyme linked immunosorbent assay (ELISA).*

Study Population And Methods: *The study done in the department of Urology and Microbiology, Government Stanley Medical College collaborated with Tuberculosis Research Centre, Indian Council of Medical Research (TRC-ICMR), Chennai, Tamilnadu, India in one year period. In a total of 135 study population, 55 clinically diagnosed GUTB patients, 43 disease control patients and 37 healthy control study groups were included. Morphological and cultural identification in pulmonary and extra pulmonary samples were done. The blood samples collected from all patients, theseparated serum samples were analyzed for the antibody response of isotype IgA and IgG to the mycobacterial cell wall purified antigens of 38KDa, 16KDa, antigen 85 complex (30KDa) and recombinant antigen 27 KDa by ELISA method.*

Results: *The IgA antibody response for antigen 85 complex (12%), 38KDa (94%), 16KDa (20%), recombinant antigen 27 KDa (38%) showed significant sensitivity compared to IgG antibody response. CONCLUSION: This study clearly showed significant sensitivity (94.54%) and specificity (98.75%) of IgA antibody response to the species specific antigen 38KDa.*

Keywords:*Nontuberculous mycobacteria, cell wall antigens, antibody response, ELISA*

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I. INTRODUCTION

Extra pulmonary tuberculosis (EPTB) constituted about 15 to 20 % of all patients of TB. [(1)] Genitourinary tuberculosis (GUTB) comprises approximately 6% of extra pulmonary tuberculosis. [(2)] Genitourinary tuberculosis continues to be a significant clinical problem because of its nonspecific clinical presentation and various radiological presentations. Early diagnosis and prompt treatment of tuberculosis is important in order to minimize the complications like, obstructive lesions,

fibrosis, contraction of orifices and finally, complete destruction. [(3, 4 ,5)]Non-tuberculous mycobacteria cause infection in immunosuppressant individuals with renal disease caused by *Mycobacterium kansasii* and *Mycobacterium avium / intra cellulare.*[(6)] The clinical features and radiological findings become more unusual in non-tuberculous mycobacterial infection.[(7)]As genitourinary tract tuberculous infection is an occult form of tuberculosis, the clinicians will be challenged greatly in their efforts to diagnosis and treatment of the patients.

The conventional laboratory diagnosis of GUTB relies on the microscopy and the culture of *Mycobacterium tuberculosis* in urine, pus, cystic fluid, and biopsy samples of genitourinary tract.Due to paucibacillary nature of extra pulmonary tuberculosis infection the clinical samples may not have adequate bacillary load and intermittent shedding of acid fast bacilli in renal tract, so it is very difficult to demonstrate the acid fast bacilli in samples. Due to intermittent shedding and collection of bacilli in bladder fluid, it is possible to demonstrate acid fast bacilli in whole early morning urine samples of GUTB.[(8)]To improve the diagnostic tests for EPTB and to increase the sensitivity and specificity, various purified and recombinant cell wall antigens were used and utilized, especially in immunological tests like ELISA. [(9, 10, 11,12)] It is found to be useful in low prevalence countries,where the positive predictive value is 93-94%, compared to high prevalence countries like India and Egypt, where the positive predictive value drops to 77-88%. [(13)]

II. Study Groups And Methods

A total of 135 patients were categorized as follows: 55 patients were diagnosed as genito urinary tuberculosis (GUTB) by clinical and radiological diagnosis in the department of urology, Stanley Medical College. Forty three Patients with signs and symptoms of other than tuberculous infectionwere included.Thirty sevenhealthycontrols were selected from various department workers after getting consent. The study was conducted in Government Stanley Medical College approved by the ethical committee of the Dr.MGR Medical University, Chennai, and the indigenous Health Committee of the Ministry of Health, Tamilnadu, India.

Three consecutive early morning whole urine samples were collected in 500 ml sterile bottle (autoclaved at 121^{O}C for 15 minutes) from all study groups.Pus, cystic fluid, and biopsy samples were collected in sterile universal container from patients who underwent the surgical intervention. The samples were examined microscopically byZiehl-Neelson and Auromine phenol staining technique. Blood samples were collected under aseptic precautions and serum were separated from all the study subjects and stored after proper labeling at –20°C for future serological work.

III. Procedure For Decontamination And Culture

Entire urine and cystic fluid specimen were distributed in 25 ml volume of sterile universal containers and centrifuge at 3000 rpm for 15 minutes.All deposits were pooled in a single container and supernatant fluid in another container. Added equal volume of 1 ml of 5% H_2SO_4 and 1 ml sterile distilled water mixed and allowed to stand for 15 minutes. Filled up the bottles with distilled water and centrifuged. Deposits and supernatant were inoculated into two sets of Lowenstein Jensen (LJ) medium containing glycerol and Kirchnner`s liquid synthetic medium (SK) containing fetal calf serum.Sputum, pus samples were homogenized and tissue samples were grinded in a tissue grinding apparatus. To 5 ml of specimens, 10 ml of 4% NaOHwas added and allowed to stand for 15 minutes at room temperature with occasional shaking, thencentrifuged at 3000 g for 15 minutes. Supernatant was separated and kept aside. 20 ml sterile distilled water was added to re-suspended sediment and centrifuged at 3000g for 15 minutes. Supernatant fluid was inoculated to two slopes of LJ and SK mediumand inoculated the deposit into two slops of LJ and SK medium. The inoculated media were incubated at 37^{O}C and observed the colony morphology, pigmentation of the growth every week. The standard bio-chemical tests-niacin test,para-nitro benzoic acid (PNB) susceptibility test, catalase at 68^{O}C/PH7.0 - were done for identification of *M.tuberculosis*. Antimycobacterial susceptibility was done byabsolute concentration (Minimal Inhibitory Concentration) method by using four anti-TB drugs such as; Rifampicin (128µg/ml), Isoniazid (5µg/ml), Ethambutol (8µg/ml), Streptomycin (8µg/ml). *M.tuberculosis* $H_{37}R_v$ was used as a standard control.

IV. Elisa

The serum samples of all study groups were analysed for antibody response of isotypeIgG and IgA to the mycobacterial cell wall purified antigens of 38KDa, 16KDa, antigen 85 complex and recombinant antigen 27 KDa by ELISA.Preparation of antigens was carried out in TRC-ICMR. M.tuberculosis $H_{37}R_V$ was grown in Sauton's Liquid medium for 6 weeks as a surface pellicle and purified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). The antigen was aliquoated 2.5 mg per ml and stored at -70°C. ELISA was carried out to estimate the IgG and IgA antibody titer against the purified proteins namely the 38KDa, 30KDa, 16KDa antigens of M.tuberculosis$H_{37}R_v$ and recombinant 27KDa antigen in various categories of patients and healthy individuals.

The samples to be assayed in a plate were randomly allocated to different wells within the plate and were also coded to conceal the identity of the specimens. In each of the experiment replicates of a positive reference serum was included. The mean value of positive reference serum in independent experiments was taken as a constant reference value to assess plate to plate and day to day variations. In spite of the differences that occurred in setting up the assays on different days and in different plates, it was possible to validly compare the optical density (OD) distributions of one category with that of another. A reference serum was included in all the plates.

Each plate had blank wells which represents the wells without antibody binding. Polystyrene ELISA plate (96 wells, NuncMaxisorp, flat bottom) were coated with 100µl of diluted (1 in 500 i.e. 5µg per ml) antigen. The plates were incubated with antigen overnight at 4^{0}C. Then the plates were washed two times with phosphate buffer saline Tween 80 (0.1% PBST) by the automated ELISA washer (OrganonTeknika), Then the non-specific sites in the wells were blocked with bovine serum albumin (1% BSA) for 1 hr at 37^{0}C and then washed the plate twice with PBST. Then 100µl of 1:200 dilution of patients and controls serum were added and incubated the plates for 1hr at 37^{0}C and the plates were washed five times with PBST. 100µl of diluted 1:2000 antihuman IgG peroxidase conjugate containing 1% BSA was added. The plates were incubated for 1 hr at 37^{0}C and the plates were washed 5 times with PBST. Then 100µl of substract (sigma 101k1242) was added and incubated for 5 minutes at 37^{0}C. Then 50µl of stopping solution (H_2S0_4) was added. After arresting the reaction,

the optical density was read in the Spectromax ELISA reader (*Molecular devices, USA*) at 490 nm wave length. IgA antibody determination was also carried out among the same patients and control subjects.Smear for AFB,LJ culture and histopathology test results were compared with ELISA method and calculatedsensitivity, specificity, positive predictive value and negative predictive values by standard statistical analysis.

V. Results

Among 135 patients, 55 patients were diagnosed as GUTB based on signs,symptoms and radiological findings suggesting genito urinary tuberculosis, 43 patients with noninfectious renal diseases other than tuberculosis and 37 normal healthy controls were included in this study. Out of 55 GUTB patients,6 patients were less than15 years, 20 patients were 15 to 40 years, 29patients were more than 40 years. In gender wise distribution, predominantly male (60%) were recorded. Among the 55 patients, 11 (20%) were positive for AFB smear and 9 were culture and smear positive. In 55 GUTB patients, 2 patients were sputum AFB smear (reported by Revised National Tuberculosis Control Program guidelines) and culture positive. Among 9 culture positive patients, 3 patients were reported as *Mycobacterium tuberculosis* confirmed by standard biochemical tests and 6 were reported for nontuberculous mycobacteria such as scotochromogen(3), photochromogen (2) and nonchromogen (1), according to the guidelines of Runyon`s classification in repeated three consecutive early morning urine samples. Out of the three*M.tuberculosis* culture positive patients, two patients were sensitive to all first line anti-tuberculosisdrugs. One patient was multi drug resistant tuberculosis, resistant to rifampicin and isonized. Two pulmonary isolates were alsosensitive to all first line antituberculous drugs.All the serum samples were subjected to ELISA test for evaluating the antibody level to mycobacterial antigens 38KDa, Ag 85 complex, 16KDa and 27KDa.Table 1(a) explained the IgA and Table 1(b) explained the IgG antibody response with the reports of smear, culture and histopathology in study subjects. Table 2 and 3 explained IgA response to mycobacterial cell wall antigens 38 KDa and 27 KDa showed more sensitivity and specificity compared with other mycobacterial cell wall antigens.Table 4 explained IgA and IgG antibody response to overall four purified mycobacterial cell wall antigens compared with smear, culture and histopathology positivesamples**.**Table 5 explained thehumoral immune response (IgA andIgG) of 38KDa antigen for the 55 GUTB patients.

VI. Discussion

Most of the GUTB patients admitted in the Hospital with the late complications of urinary tract like obstruction, hydronephrosis, and pyelonephritis usually result in the loss of vital organ functions. In one series, (Simon HB, et al.,) describing 41 patients of genito urinary tuberculosis observed from 1962 through 1974, concomitant pulmonary findings were present in only 66% of newly diagnosed patients of genitourinary tuberculosis.[(14)]In the same series, dysuria (34%), hematuria (27%) flank pain (10%) and pyuria 5% were the most frequent symptoms for active urinary tuberculosis. however, patients may be asymptomatic and the disease discovered only after severe destructive lesions of the kidneys have developed. Urinalysis gives abnormal results in 90% of patients, revealing hematuria and pyuria. The above study also showed a positive skin test with purified protein derivative was present in 95% of patients, and urine culture grew *M.tuberculosis* in 90%, excretory urograms were abnormal in 93% of patients were examined. The Present study included 135 patients observed in one year. Clinically proved GUTB (according to clinical signs, symptoms and radiographic studies) were 55 and a control group of 80 (Disease control 43 and healthy controls 37). Most frequent presenting symptoms in the present study were dysuria (40%), flank pain (41%) hematuria (14%), pyuria (1%), constitutional symptoms (21%) and with multiple symptoms of dysuria and hematuria (7%), dysuria, hematuria and Flank pain (2%), flank pain and pyuria (1%) and other genital diseases such as prostatitis, urethral fistula involvement in 3 patients.

The following factors couldbe attributed as reasons for lower number of culture positivity in isolation of GUTB. It may be due to paucibacillary nature of the disease despite the usage of a liquid enrichment media such as Kirchner's synthetic liquid media, hardly any specimen with few organisms turned out to be positive. In 28% of patients were already started onantituberculosis therapy (ATT) on the basis of clinical, radiological and histopathology evaluation.Even though demonstration of acid fast bacilli in clinical specimen by microscopic method is very fast and reliable method, it also has certain limitations and low specificity and sensitivity. ZN staining is positive if the number of *M.tuberculosis* is more than 10 ^{4}/ml of specimen.[(15)] Instead of ZN staining,fluorochrome stained smears can be used for easier detection of organisms against background and significantly larger area of the smear can be screened per unit of time [(16)] but it has certain limitations like cost factors andlabour intensive. Though demonstration of mycobacterium in various clinical specimens remains the gold standard, this often not possible in extra pulmonary tuberculosis due to paucibacillary nature. The yield of culture varies from 30-50%.Higher yields up to 70 % have been reported in infants.[(17)]Demonstration of host's response to exposure to M.tuberculosis is one of the diagnostic tool with limited sensitivity and specificity. Humoral immune response may be assessed by measuring the immunoglobulins to various antigens in patients' blood sample.Enzyme linked immunosorbent assay has been used to detect antibodies to various purified or

complex antigens of M.tuberculosis. Few studies were reported by demonstration of elevated level of purified cell wall mycobacterial antigens to specific antibodies (IgG, IgA andIgM). A variety of antigens have been adapted for the serodiagnosis of TB. 38KDa antigen which is a phosphate- binding protein, was reported to be species specific to the M.tuberculosis complex. This antigen has been identified as a potential reagent to be used for the screening of TB. [18] Zhou et al. reported 38 KDa antigen showed greater sensitivity (89%) and specificity (93%) in extra pulmonary tuberculosis patients.[19] Specificities of the test reported previously coinciding from 88-100%, but the reported sensitivities of the test vary 33-89% in smear positive TB patients and 16-54% for smear negative TB patients [19,20,21,22,23and 24). Although antibody response to the 38Kda antigen in pulmonary TB has been extensively studied, there are only few reports about utility of the 38 KDa-based serological tests in extra pulmonary TB. Antibodies were detected in 12-56% of extra pulmonary TB patients. [25]Wilkins and Ivanyi[26 and 27] described competition ELISA assays based on the high sensitivity and specificity of the 38 KDa monoclonal antibody. Antibodies were detected in 73% of patients of extra pulmonary TB at a chosen specificity of 98%. The TB patient does not produce antibodies against all antigenic substances present in the cell wall of the tuberculous bacilli, and the specificities of the antibodies differ among patients.

Lyashenko et al. [28] pointed out that person to person variation of antigen recognition, rather than recognition of particular antigens, is a key attribute of humoral immunity in human TB. Heterogeneity of antigen recognition by serum antibodies during TB explains the failure to detect specific antibody response in TB patients when only a few purified antigens of M.tuberculosis were used. [29]In our study we pointed out smear and culture positive patients were showed notable level of specific IgA antibody response against 38 KDa and 27 KDa antigens than IgG response to these antigens. In addition the interesting and significant factor in this study was smear and culture negative patientswere alsoshowed considerable level of specificity and sensitivity of IgA response to 38 KDa and 27 recombinant antigens than IgG antibody.

VII. Conclusion

Variability of humoral immune responses to the mycobacterial antigen proves that the antibody response to a particular antigen may not be universal. The antibody response to the 38KDa antigen in pulmonary TB has been extensively studied but few reports shown the utility of the 38 KDa based serological tests in extra pulmonary TB.We carried out this study in genitourinary tuberculosis and its humoral immune response to four different purified mycobacterial cell wall antigens. We have derived the higher sensitivity and specificity of the IgA response to 38 KDaantigen, next high level of response to 27KDa.The valuable finding in this study was the smear and culture negative but clinically proved GUTB patients were showed high level of specificity and sensitivity of IgA response to 38KDa and 27KDa antigens.

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References:

[1] Fanning A. Tuberculosis: 6.Extrapulmonary Disease. CMAJ 1999; 160:1597-603.
[2] LubbeJ,Ruef C, Spring W, Dubs M,Sigg C. Infertility as the first symptom of male genitourinary tuberculosis. Uroint 1996; 56(3):204 -6.
[3] Christenson, WI. Genitourinary tuberculosis Review of 102 cases. Medicine 1974; 53:3.
[4] Lavender, J.P. Hypertension and tuberculous renal lesions Br. Med 1957;1: 1221
[5] Lieherthal, F. Renal tuberculosis, the development of the renal lesion, Surg.Gynecol.Obset1938; 67: 26.
[6] Meddler, E.M .cases of renal infection in pulmonary tuberculosis. Evidence of healed tuberculosis lesions AM . J. Pathol 1963; 2: 401.
[7] Merck, J.J Karlson, A.G., and Carr, D.T. Diseaes in man associated with unclassied acid fast bacteria. Mayo Clin.Pro 1963; 38: 271
[8] Mitchison, D.A. And Aber, V.R cultures of specimens other than sputum for mycobacteria. Journal of clinical microbiology1974; 27: 883,.
[9] Julian E,MatasL,HernandezA,AlcaideJ.LuguinM.Evaluation of a new serodiagnostic TB test based on immunoglobulin A detectection against Kp-90 antigen. Int.J.Tuberc.Lung Dis 2000; 4:1082-1085.
[10] Chan ED, Reves R, Belisle JT, Brennan P, Hahn we.Diagnosis by visually detectable immunoassay for lipoarabinomannan.Am J RespirCrit Care Med 2000; 161:1713-1719.
[11] Harrington JJ 3rd, HoJL, Lapa e Silva JR etal.Mycobacterium TB Lipid antigens: use of multiantigen based enzyme immunoassay for free and complex dissociated antibodies. Int.J.Tuberc.Lung Dis 2000; 4:161-167.
[12] Pottumaruthy S, Wells VC, Morris AJ. A comparison of seven tests for serological diagnosis of TB.J.ClinMicrobiol 2000; 38:2227-2231
[13] Ahmad, A.Afghan, S., Raykundalia, C.andCatty,D.Diagnosis of tuberculosis by using enzyme linked immunosorbent assay (ELISA) to detect antimycobacterial superoxide dismutase in the patients.J Islamic Acad of Sci 11:1, 1998.
[14] Simon HB, Weinstein AJ, Pasternak MS et al: Genitourinary tuberculosis: clinical feature in a general hospital population. Am. J Med 63:410.1977.
[15] Allen BW Mitchison DA. Counts of viable tubercle bacilli in sputum related to smear and culture gradings. Med Lab Sci 1992:49.

[16] Strumpf IJ, Tsang, AY, Sayre JW.Reevaluation of sputum staining for the diagnosis of pulmonary tuberculosis. Am Rev Respir Dis 1979; 119:599-602.
[17] Vallejo J, Ong LT, Starke JR. Clinical features, diagnosis and treatment of tuberculosis in infants. Pediatrics 1994; 94:1-7.
[18] Pfyffer, G; Brown – Elliot, BA; Wallace, WC; Jr. Mycobacterium: General characteristics, staining procedures. In: Murray PR, Baron EJ, Jorgensen JH,PfallerMA,Yolken RH., editor. Manual of clinical microbiology.8.Vol.1.Washington, DC: asm Press; 2003.pp.532-559.
[19] Zhou AT, Ma WL,ZhangPY,ColeRA.Detection of pulmonary and extra pulmonary tuberculosis patients with the 38Kilodalton antigen from mycobacterium tuberculosis in a rapid membrane based – assay. ClinDiagn Lab Immunol 1996; 3: 337-341.
[20] Silva VMC,Kanaujia G, GennaroML,Menzies D . Factors associated with humoral response to ESAT-6, 38 Kda and 14 Kda in patients with a spectrum of tuberculosis. Int J Tuberc Lung Dis 2003; 7:478-484.
[21] Wilkins EGL Antibody detection in tuberculosis.In: Davies PDO, ed. Clinical Tuberculosis. London, Chapman & Hall, 1998; pp.81-96.
[22] Bothamley GH. Serological Diagnosis of tuberculosis.EurRespir J 1995; 8:Suppl. 20 676s-688s.
[23] Chan ED, Heifers L, IsemanMD.Immunologic diagnosis of tuberculosis: A review Tuber Lung Dis 2000; 80:131-140.
[24] Menna LS,GoelS,ShamaSK,et al. Comparative study of three different mycobacterial antigens with a novel lipopolysaccharide antigen for the serodiagnosis of tuberculosisClin Lab Anal 2002;16:151-155.
[25] Wilkinson RJ, Haslov K, RappuoliR,et al. Evaluation of the recombinant 38 Kilo Dalton antigen of Mycobacterium tuberculosis as a potential immunodiagnostic reagent. J ClinMicrobiol 1997; 35:553-557.
[26] Uma Devi KR,Ramalingam B, Brennan PJ,Narayanan PR, Raja A.Specific and early detection of IgA, IgG&IgM antibodies to Mycobacterium tuberculosis 38 Kda antigen in pulmonary tuberculosis.Tuberculosis (Edinb) 2001; 81:249-253.
[27] Wilkins EG, Ivanyi J. Potential value of serology for diagnosis of extra pulmonary tuberculosis. Lancent1990; 336: 641-644.
[28] .Lyashchenko K,Colangeli R, HoudeM,AlJahadiH,Menzies D, Gennaro ML. Heterogeneous antibody response in tuberculosis. Infect Immun1998; 66:3936-3940.
[29] IvanyiJ,Bothamely GH, Jackett PS. Immunodiagnostic assays for tuberculosis and leprosy. Br Med Bull 1998; 44:635-649.

Table 1(a). IgA antibody response compared with smear, culture positive and smear, culture negative patients.

Conventional tests results (N=55)	IgA Response Positive cases	Negative cases	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
AFB Smear alone positive culture negative(11)	7	4	63.63	98.75	87.5	95.18
Smear and culture positive(9)	6	3	66.66	98.75	85.71	96.34
Histopathology positive (2)	1	1	50	98.75	50	98.75
Smear and culture negative (33)	13	20	39.39	98.75	92.85	79.79

Table 1(b).IgG antibody response compared with smear, culture positive and smear, culture negative patients.

Conventional tests results (N=55)	IgG Response Positive cases	Negative cases	Sensitivity	specificity	PPV (%)	NPV (%)
AFB Smear alone positive culture negative(11)	6	5	54.54	98.75	85.71	94.04
Smear and culture positive(9)	3	6	33.33	98.75	75	92.94
Histopathology positive (2)	0	2	0	98.75	0	97.35
Smear and culture negative (33)	4	29	12.12	98.75	80	73.14

Table2. IgA antibody response to selected purified cell wall mycobacterial antigens for the study.(GUTB=55, Control=80).

Cell wall antigens	IgA positive response	IgA negative response	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
38 KDa	52	3	94.54	98.75	98.11	96.34
85 KDa	7	48	12.72	100	100	62.5
16 KDa	11	44	20	98.75	91.66	64.22
27 KDa	21	34	38.18	98.75	91.30	69.11

Key:**KDa- Kilodalton, PPV- Positive predictive value, NPV- Negative predictive value**

Table 3.IgG antibody response to selected purified cell wall mycobacterial antigens for the study. (GUTB=55, CONTROL=80)

Cell wall antigens used	IgG positive response	IgG negative response	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
38 KDa	13	42	23.63	100	100	65.57
85 KdDa	1	54	1.81	100	100	59.70
16 KDa	12	43	21.81	100	100	65.04
27 KDa	3	52	5.45	98.75	75	60.30

Table 4. IgA and IgG antibody response to overall four purified mycobacterial cell wall antigens compared with smear culture and histopathology positive cases.

S + C+ HP	IgA Response No.of positive cases	No.of negative cases	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	IgG Response No.of positive cases	No.of negative cases	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
N=22	14	8	63.63	96.25	82.35	90.58	9	13	40.90	96.25	75	85.55

KEY : S – Smear , C – Culture , HP - Histopathology

Table: 5 Humoral immune response (IgA &IgG) of 38KDa antigen for the following patient categories (N=55):

Patient's categories.	No.of cases	IgA response to 38KDa antigen	IgG response to 38KDa antigen
Clinical suspicion of GUTB with complication of genitourinary system	55	52	13
Concomitant pulmonary findings (X-ray result / smear positive/culture positive)	2	2	2
Proven pulmonary TB, who were treated fully already	5	4	2
Histopathology positive for GUTB	2	1	1
Post primary complex & treated with INH and RIF	1	1	1
Mantoux positivity (including urine smear positive 1 cases, sputum smear positive with proved pulmonary lesions in chest X-ray – 2 cases).	23	19	13
Patients started recently with ATT	15	9	5
Only Abnormal X-ray report suggestive of tuberculosis	33	28	19
Smear for AFB +ve(9) / culture +ve (typical)(3)/culture +ve (Atypical)(6)	9	6	3
AFB smear alone +ve	11	7	4