Rapid Detection of Tuberculosis using Urine Samples: Development & Validation of ELISA Technique

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***Abstract*: Diagnosis of tuberculosis (TB) and its early detection can be possible only when an individual permits itself for early diagnosis. In the recent study, the serum elevated against *Mycobacterium tuberculosis* antigen was settled for the diagnosis of tuberculosis infection employing serological tests. The mycobacterial proteins was identified based on indirect ELISA including high sensitivity of 0.1 mg and the protein ranging from 20 kDa to 150 kDa exhibit immunoreactivity in western blotting applying TB serum. Correlation coefficient among various phases of infected urine samples assemble from different regions of Odisha with the titres of Ab was seized as the primary end point for the regularity of protocol. (Maximal correlation r = 0.999) was detected in phase III infected urine sample assemble from Mayurbhanj, whereas minimal low correlation along with TB serum (r = 0.703) was marked in phase I infected urine sample assemble from Balasore. (Linear positive correlations R^2) present within antigenic proteins at all the phases with TB serum. Henceforth, the TB serum could be used for the early detection of TB. This immuno serological accesses us to design a robust, highly sensitive & specific with accuracy diagnostic kit for initial detection of tuberculosis infection.**

***Key words*: Tuberculosis, Serological test, Immune-reactivity, Correlation coefficient, TB serum, Early detection, Diagnostic kit**

I. INTRODUCTION

Tuberculosis (TB), one of the oldest recorded human diseases, stands as the considerable killers among all other contagious and transmittable diseases, even though the universal use of a live constricts vaccine and certain antibiotics. According to the survey, TB has been re-emerged globally as a result of the evolution of drug and multi drug resistant strains (Shah et al., 2007., Palomino et al., 2002). *Mycobacterium tuberculosis* (*Mtb)* is an aerobic and acid fast bacilli causing TB, remains dormant state for many years in host's body beyond provoke any symptoms or spreading, however the immune system inclines into depleted, mycobacteria eventually be effective that infects overall the lungs along with other parts of body. In addition, TB concern is also aggravated by alternative ailments that disturb the immune system, such as HIV, frequent in developing countries.

Despite of constant attempts from worldwide, only confined achievement has been concluded in detection and disease diagnosis of TB (Nahid et al., 2006., WHO., 2014). Previously, *Mycobacterium tuberculosis* culture remains the gold standard for TB diagnosis. Developing countries having poor living condition, people are still facing resource-poor settings that mainly outbreaks the TB transmission which results constantly increasing of mortality rate.

Another major aspect is the high population of HIV bearers in these places having increasing risk of developing active tuberculosis from *Mycobacterium tuberculosis* infection (Davies and Pai 2008). Apart from these factors, there are many other obstacles that face successful field detection and treatment of TB like rising costs of the first line anti-TB drugs (isoniazid, rifampicin, pyrazinamide, ethambutol) for TB treatment to suppress resistance (Mitnick et al., 2003., Caminero, 2006., Isaakidis et al., 2011). To date, most diagnostic methods like immunoassays are not having high specificity towards TB. As some Mycobacterium species also shares antigens with *Mycobacterium tuberculosis* eventualized false positive cases in 35% of active TB infected patients (Colijn et al., 2011., Bekmurzayeva et al., 2013., Somoskovi et al., 2000., Singh et al., 2012). Other techniques like flowcytometry, radiometric detection, latex agglutination etc. hold their own set of imperfections.

However, some studies show limelight on various approaches for detection of tuberculosis antigenic specific biomarker from urine but no trustworthy attempt has been made so far. Therefore, some attempts has been made; (a) to characterize the distinct steps for the advancement of the serological methods for the revelation of *Mycobacterium tuberculosis* infected urine samples assemble at distinct phases of infection, (b) to calculate the (specificity & sensitivity) of the serum against antigen & to resolve even if antibody capture depend on the detection of *Mycobacterium tuberculosis* employing the serum at distinct phases of infection was viable; (c) to estimate and compare the correlation coefficient studies for various stages of TB infection development and

raise the possibilities for rapid detection of mycobacterial antigenic marker in urine at the initial phase of tuberculosis.

II. METHODOLOGY

Assortment of infected Tuberculosis urine and blood:

Mycobacterium tuberculosis (Mtb) infected urine (an early morning or spot urine samples, approx. 200 ml each) and blood samples were collected from different districts Head Quarter Hospitals of Odisha (Balasore, Bhadrak, Cuttack, Jajpur, Khorda and Mayurbhanj). Total 50 positive TB urine samples were aliqouted and kept in -80^{o} C. The collected urine samples were classified into three phase of TB infection (Mild, Acute and Chronic) based on their antigenic conc. The protein conc. of 50 positive mycobacterial infected urine samples were shown in table 1.

Table 1. Urine sample collection from different places of Odisha and total concentration of protein (mg/ml) in urine-TB infected urines at various phases of TB infection.

Places of sample Collection	**No of samples collected**	**Sample Codes**	**Conc. of antigenic (*Mycobacterium tuberculosis*) Proteins (mg/ml)**		
			Mild	Acute	Chronic
Balasore	7	TBUS1	0.215±0.006	4.169±0.039	4.852±0.031
		TBUS2	0.167±0.020	3.089±0.017	4.150±0.044
		TBUS3	0.236±0.025	4.429±0.020	5.026±0.027
		TBUS4	0.152±0.071	3.962±0.055	4.436±0.056
		TBUS5	0.268±0.051	4.527±0.078	5.319±0.095
		TBUS6	**0.114±0.005**	3.998±0.101	4.665±0.040
		TBUS7	0.187±0.011	4.032±0.020	4.852±0.033
Cuttack	4	TBUS8	0.219±0.012	4.675±0.109	5.153±0.061
		TBUS9	0.138±0.016	4.272±0.042	5.12±0.017
		TBUS10	0.215±0.010	4.560±0.020	5.399±0.143
		TBUS11	0.166±0.010	3.82±0.014	4.964±0.057
Bhadrak	12	TBUS12	0.204±0.007	4.313±0.010	5.652±0.018
		TBUS13	0.154±0.021	4.157±0.035	4.928±0.022
		TBUS14	0.513±0.013	4.207±0.008	6.356±1.067
		TBUS15	0.315±0.011	4.745±0.013	5.644±0.014
		TBUS16	1.337±0.012	8.796±0.006	9.663±0.011
		TBUS17	1.016±0.010	8.022±0.012	8.991±0.009
		TBUS18	0.965±0.023	6.189±0.010	7.049±0.025
		TBUS19	0.431±0.015	5.285±0.016	6.666±0.020
		TBUS20	0.306±0.010	4.525±0.020	5.727±0.028
		TBUS21	0.185±0.015	3.718±0.020	4.848±0.034
		TBUS22	0.687±0.015	5.464±0.082	6.924±0.040
		TBUS23	0.901±0.023	6.078±0.126	7.189±0.037
Jajpur	9	TBUS24	1.092±0.066	8.553±0.007	9.411±0.010
		TBUS25	0.951±0.003	6.02±0.010	6.926±0.030
		TBUS26	0.811±0.010	6.241±0.051	6.997±0.017
		TBUS27	1.943±0.057	8.953±0.061	9.958±0.068
		TBUS28	1.635±0.073	8.736±0.089	9.562±0.110
		TBUS29	1.076±0.064	7.075±0.061	7.911±0.029
		TBUS30	0.622±0.059	5.348±0.113	6.236±0.015
		TBUS31	0.965±0.066	6.287±0.055	7.058±0.041
		TBUS32	0.250±0.021	3.971±0.157	4.747±0.221
Mayurbhanj	15	TBUS33	0.839±0.090	6.405±0.146	7.350±0.115
		TBUS34	0.460±0.053	5.061±0.047	5.834±0.110
		TBUS35	2.672±0.361	9.28±0.188	10.098±0.090
		TBUS36	1.736±0.151	7.804±0.320	8.720±0.145
		TBUS37	1.318±0.088	7.851±0.167	8.663±0.188
		TBUS38	0.856±0.121	6.110±0.098	6.727±0.214
		TBUS39	0.744±0.022	5.469±0.095	6.546±0.177
		TBUS40	2.454±0.067	9.556±0.145	**10.314±0.281**

		TBUS41	0.667±0.158	5.207±0.225	5.642±0.317
		TBUS42	0.871±0.131	6.119±0.188	7.121±0.074
		TBUS43	0.299±0.031	3.850±0.120	4.646±0.098
		TBUS44	0.652±0.084	5.460±0.132	6.862±0.106
		TBUS45	0.381±0.027	4.241±0.109	5.116±0.113
		TBUS46	1.475±0.101	7.941±0.076	8.709±0.037
		TBUS47	2.182±0.099	9.084±0.076	9.752±0.104
		TBUS48	0.674±0.101	5.226±0.112	6.192±0.064
Khordha	3	TBUS49	1.181±0.078	7.567±0.061	8.449±0.265
		TBUS50	0.402±0.164	4.410±0.096	5.579±0.177

Mean±Standard Deviation; n=3

Mild, Acute, Chronic represent three phases of infection.

Mild (Stage 1): Asymptomatic phase of infection. Lowest protein content is mentioned in **(bold); TBUS6**

Acute (Stage 2): The intermediate phase of infection characterized by severe coughing, chest pain, chest X-ray report and tuberculin skin test. Chronic (Stage 3): The last stage of infection characterized by severe blood coughing with high fever, positive tuberculin skin test and Chest X-ray. Highest protein content is mentioned in **(bold); TBUS40**

Optimization of Mtb proteins:

The absolute protein content of TB infected urine samples were optimized by dye binding method i.e.; Bradford method for protein estimation (Bradford, 1976) and using Bovine Serum Albumin (BSA) as a standard, with known concentration of 2 mg/ml.

Mycobacterium tuberculosis antigenic proteins separation by SDS-Polyacrylamide gel electrophoresis:

Mycobacterial antigens was got separated by 12% SDS-PAGE consist of proteins conc.ranging from 0.114 mg/ml (mild) to 10.314 mg/ml (chronic), employing a (Bio-Rad Mini-Protean vertical electrophoresis) (Laemmli, 1970). For the determination of molecular weight of the antigenic proteins, a standard protein ladder was also run alongside with the samples. Separation of proteins hold executed under a volt of 120 V (constant). Further, staining of gels was done with Coomassie Brilliant Blue (CBB- R 250 Sigma) for the visualization of mycobacterial antigenic proteins and the protein ladder.

Screening of TB infected urine by Indirect ELISA:

All 50 TB infected urines with respective serums along with 5 healthy controls and 10 negative controls were tested by indirect ELISA. Healthy urine samples were used as negative controls and other proteins (negative to TB) as positive controls. The serum was diluted with dilution buffer (1% skimmed milk in 1X Phosphate Base Saline Tween 20) in the ratio of 1:2500. The antiserum was coated with 100µl of 1X coating buffer (GeNei^{TM,},Mt. Hope, Ohio, U.S.) and kept for 12hr at 4^{o}C. After 24hr, wash the plates for three times with 1X Phosphate Base Saline Tween 20 (PBST) and blocking buffer (2.5% skimmed milk with 1X Phosphate Base Saline Tween 20 (PBST) was mixed & kept for 1 hr at RT. Next, washed ELISA plates with 1X Phosphate Base Saline Tween 20 (PBST) was then diluted with 100 µl serum & added to each well. After 12 hr of incubation at 4^{o}C again the ELISA plates were washed with 1X PBST. Then 100µl of diluted horseradish peroxidase-conjugated anti-human IgG (Bio-Rad) was added to each well at a dilution of 1:10000, kept for 1 hr at RT. After washing of the ELISA plates for four times with 1X Phosphate Base Saline Tween 20, 200µl of TMB/H_2O_2 substrate was added to individual well and an optimal density from the color evolved was calculated at the wavelength (450 nm) on ELISA reader (mindray MR-96A).

Analysis by Western Blotting:

Mycobacterial antigens that are used in approximate estimation were then adjusted by accumulating equivalent volumes of 50 positive samples from each phase. The separated antigenic proteins from SDS-PAGE were relocated in polyvinylidene fluoride (PVDF) membrane (millipore) of mini trans blot cell module (Bio-Rad). Immunoblotting was carried out for 1 hr 30mins at a 60 Volt. The stained Polyvinylidene Fluoride (PVDF) Membrane was done by amidoblack (Sigma) and destained with 10% methanol and 10% acetic acid. Indefinite sections of membrane strips containing the separated antigens were blocked by blocking buffer with composition of Tris Base Saline Tween-20 (TBST: 0.02 M Tris-HCL, 0.15 M NaCl, 0.05 % Tween-20 at pH 8) and consist of 3% bovine serum albumin (BSA). The PVDF membrane segment were washed with TBST buffer & kept with 1:2500 dilution of serum constructed against the mycobacterial protein for 3 hr at RT. After incubation, the PVDF membrane segments/strips was washed three times & kept for 1 hr followed by added 1:10000 dilution of HRP-conjugated anti-human IgG (Bio-Rad) at RT. The segments were washed vigorously for 20 mins with TBST, then for HRP, the substrate (Pierce ECL western blotting substrate, Thermo Scientific) was added to the segments & the delineated bands was then developed by (Hyper-Film , Kodak with developer & fixer) in the dark room. Dilutions were prepared by using 1% Bovine Fetal Serum in (TBST buffer).

III. RESULTS AND DISCUSSION

Absolute protein estimation of infected samples:

The proteins from TB infected urine samples at distinct phases were evaluated and correlated with the protein contented of

normal urine. The isolated protein from the infected urine sample was raised as an indicative higher than the normal urine samples shown in table 2.

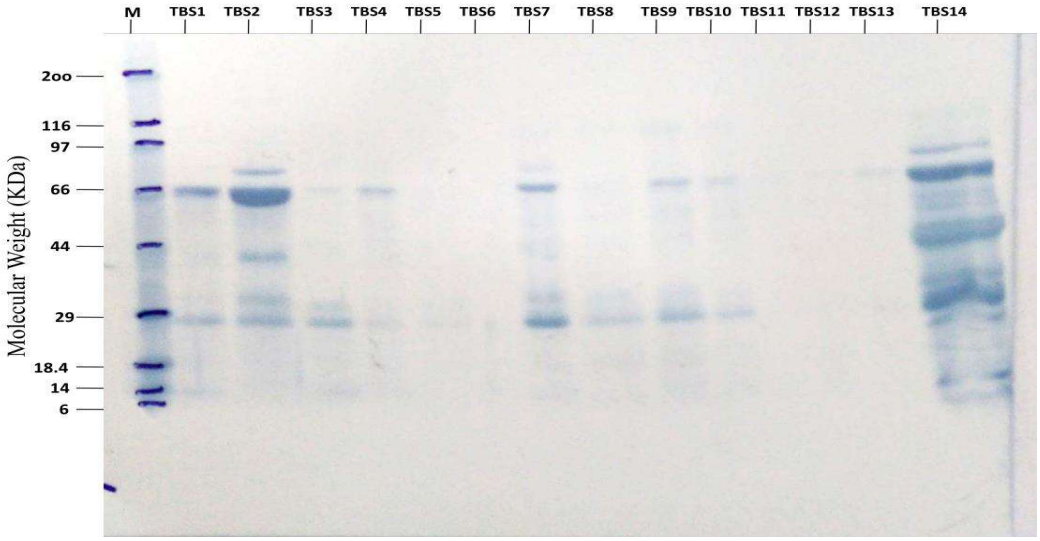
Table 2: Shows the total antigenic protein content (mg/ml) in normal and negative urine samples at different phases of TB infection.

Samples	Total Proteins
Healthy Control	-0.055±0.505
Mild infection	0.765±0.631
Acute infection	5.777±1.785
Chronic infection	6.693±1.766

Individual value stands as average triplicates samples.Values in parenthesis show the standard deviation (SD).

Gradient SDS-PAGE analysis:

A gradient SDS-polyacrylamide gel electrophoresis methodology was employed to obtain *Mycobacterium tuberculosis* proteins containing 0.1-10mg/ml. The protein separation by SDS-PAGE ensue by coomassie brilliant blue stain that shows the recognition of collective visible protein bands as shown in figure.1, with molecular weight ranges from 20-150 kDa. The bands containing respective integer and intenseness of the isolated antigenic protein from phase III TB infected urine sample were observed as a greater in similarity than phase I & II infected samples.



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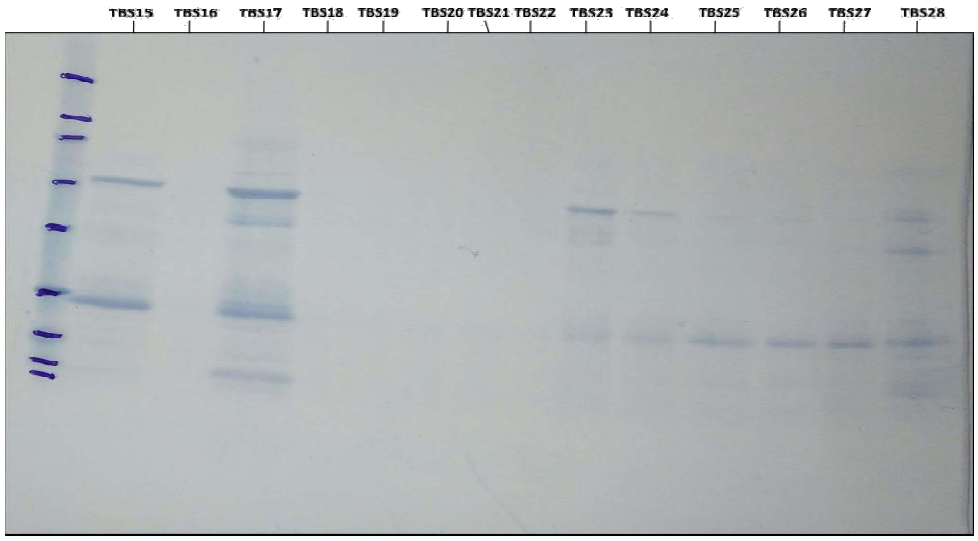

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Fig.1. Banding pattern of 12% SDS-PAGE (A) Proteins indicate from positive urine samples of phase III; (B) Proteins indicate from positive urine samples of phase II; (C) Proteins indicate from early phase of infection and from TBS36- TBS42 are normal healthy urine samples that indicate no banding pattern. Molecular weight of standard protein is shown on left margin.

Analysis of Indirect ELISA:

The primary antibody was approved at 1:1000, 1:2500 and 1:5000 adjacent to different mycobacterium protein concentration as shown in Figure.2. The analysis shows such the serum hold proficient adequate to identify up to 0.1 mg of mycobacterial protein at 1:5000 dilution. The consistent preliminary settings were preferred for the further experiments and 1:2500 antibody dilutions and 0.22 mg of antigen in individual well with 1.129 absorbance unit was confirmed as associating value. The TB serum likewise exhibited cross-reactivity reaction with antigenic proteins isolated from non TB patient urine samples, and adaptability was found to be distinctive.

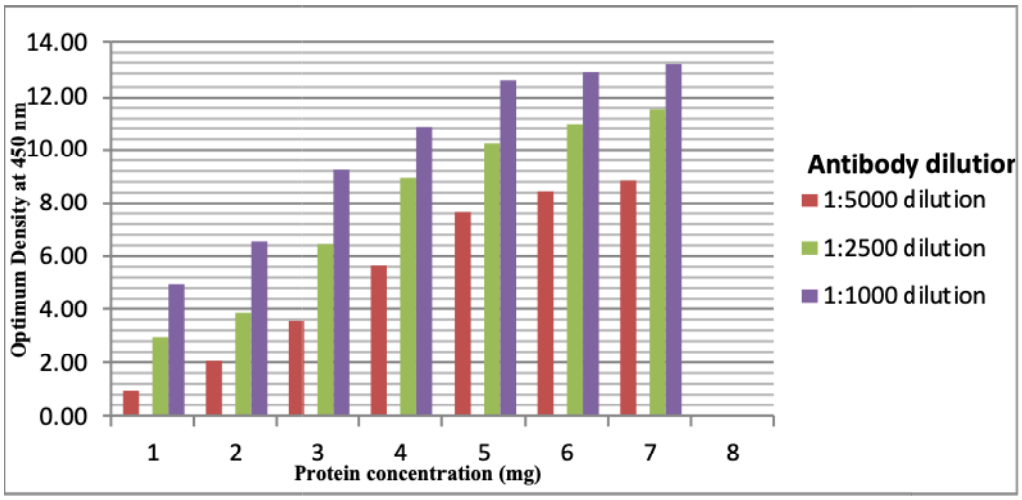


Fig 2. Indirect ELISA shows the deviation in absorbance at 450 nm as an activity of protein by employing different dilutions of the Ab as determined. Mean ± SD; n = 3.

Western Blotting Analysis:

From the analysis of western blot, antibody adjacent to the antigenic proteins at different phases of disease demonstrates exceptional characteristics in the detection profile. The response between Ag-Ab was identified against ranges between molecular weight of 10-120 kDa in all phases. Antibody responsesact immense against the antigenic proteins with molecular weight of 35 kDa, 66 kDa, and 110kDa and observed with additional bands of susceptibility, along with 14 kDa, 16 kDa, 190 kDa and 110 kDa shown in Figure 3.

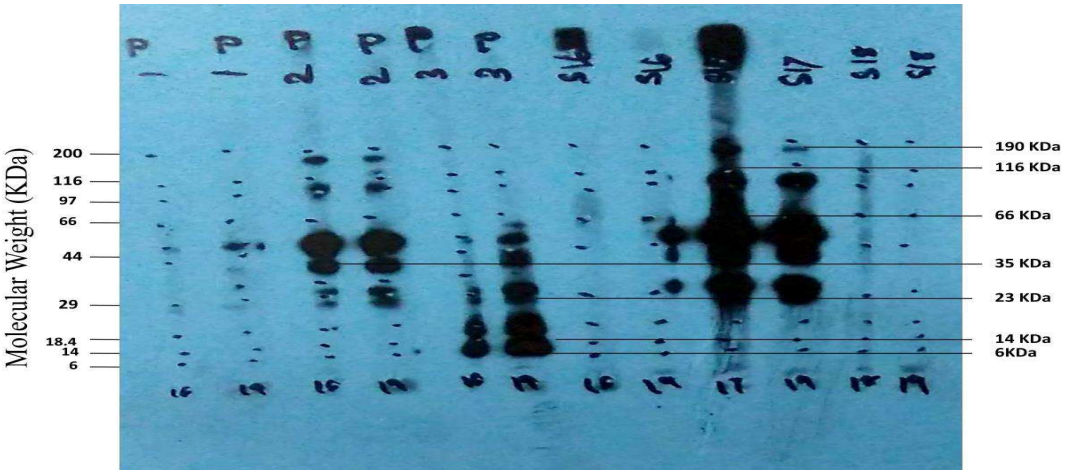
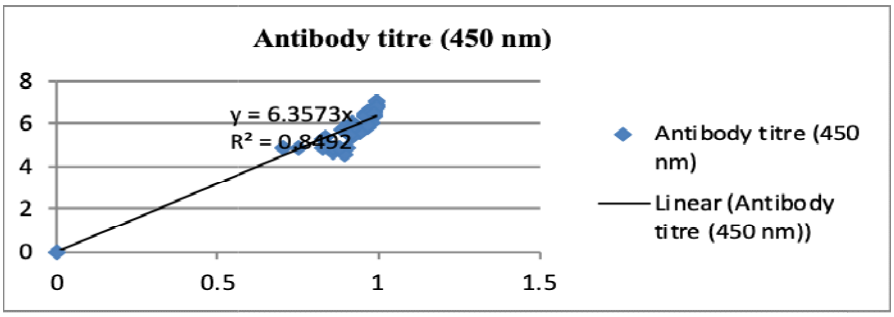


Fig.3. Analysis of Western blotting of antibody to pooled mycobacterial antigenic proteins from various phases of TB infection. Electrophoresis separated antigenic proteins were blotted onto a PVDF membrane and incubated with TB serum. The response of Ab-Ag was seen against ranging from MW of 10-120 kDa in all phases. The Ab response was high against the antigens with MW of 35 kDa, 66 kDa, 110 kDa and seen additional bands of reactivity, including 14 kDa, 16 kDa, 190 kDa and 110 kDa. Molecular weight (MW) of standard protein is shown on left margin.

Interrelationship of antigens concentration of distinct phases of TB infection and Ab titres:

Correlation coefficient relation among concentrations of mycobacterial proteins ofvarious phases of infection collected from various places of Odisha was analyzed against antibody titre shown in figure 4. Affirmative correlation ($P < 0.01$) obtains among absorption of mycobacterial antigens & Ab titre. The protein concentration of mycobacterial antigens collected from chronicphase of infection shows highest positive correlation of determination ($R^2 = 0.907$), whereas approximately lowest positive correlation of determination ($R^2 = 0.849$) estimated between the conc.of antigens of mild phase of infected samples and Ab titre. In distinct to the results, we can conclude a notable characteristicin the antigenic protein load at mild phase infected samples as correlated with other phases of infection. The antigenic protein concentration of infected samples collected from chronic phase shows coefficient of determination ($R^2 = 0.872$) which concludes then high possibilities of early detection of infection.



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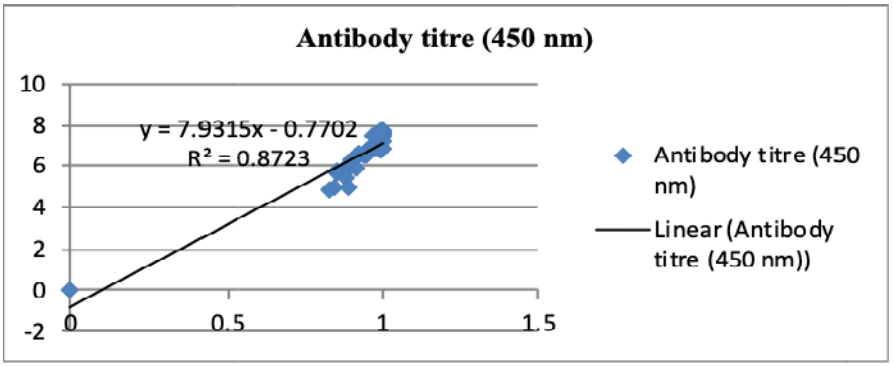

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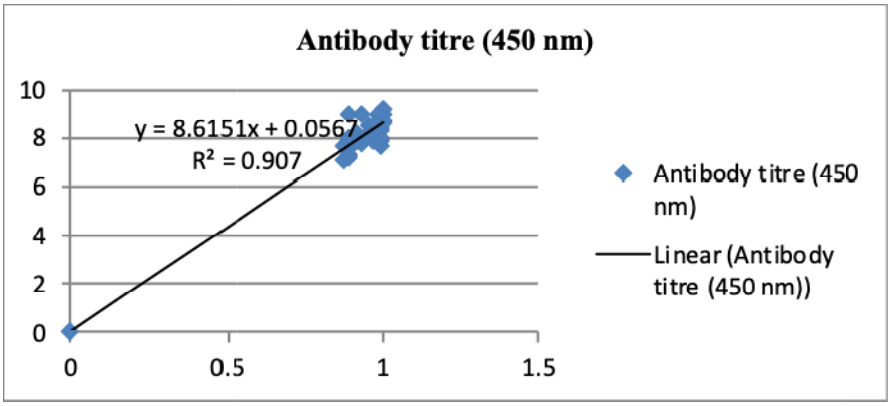

C

Fig.4.Graphical scatter plot shows linear correlation between antigenic protein conc. of different phases of infection and Ab titres. A single dot indicated as a single sample. (A) Proteins conc. collected from phase I (mild) positively correlated with Ab titres ($R^2 = 0.849$, $P < 0.01$) was measured by linear regression analysis. (B) A similar positive correlation of proteins conc. collected from phase II (acute) positively correlated with Ab titres ($R^2 = 0.872$, $P < 0.01$) and (C) Proteins conc. of phase III (chronic) shows positively correlated with Ab titres ($R^2 = 0.907$, $P < 0.01$) were measured.

Interrelationship among antigenic protein of phase I (mild) infected samples with Ab titres:

At mild phase of infection, significantly an affirmative correlation noticed obtained among the concentration of mycobacterial proteins and TB Ab titres. The TB serum indicated a positive correlation including the antigenic protein conc. of phase I infected samples. Highest positive coefficient of correlation (r = 0.999) was found in infected urine samples assemble from Mayurbhanj district of Odisha, along with TB serum was shown in table 3. Among the samples isolated from phase I and phase II of TB infection, a very minimal and negligible variation was observed. This insignificant difference might be due to transformation in the mechanism of mycobacterium.

Table 3. Pearson correlation coefficient (r) in-between concentrations of *Mycobacterium tuberculosis* antigens isolated from collected urine samples of various phases of TB infection with Ab titres obtained from distinct places of Odisha.

Sl.No	Different places of Odisha	No. of samples	Sample code	Conc. of phase I infected urine samples with the titres of Ab	Conc. of phase II infected urine samples with the titres Ab	Conc. of phase III infected urine samples with the titres of Ab
1.	**Balasore**	7	TBUS 1	0.919	0.989	0.999
			TBUS 2	0.833	0.993	0.984
			TBUS 3	0.984	**0.998**	0.998
			TBUS 4	0.703	0.984	0.987
			TBUS 5	0.900	0.990	0.992
			TBUS 6	0.752	0.952	0.976
			TBUS 7	0.997	0.994	0.989
2.	**Cuttack**	4	TBUS 8	0.968	0.935	0.962
			TBUS 9	0.969	0.963	0.981
			TBUS 10	0.965	0.992	0.978
			TBUS 11	0.979	0.917	0.884
3.	**Bhadrak**	12	TBUS 12	0.943	0.997	0.991
			TBUS 13	0.861	0.880	0.892
			TBUS 14	0.885	0.856	0.874
			TBUS 15	0.962	0.919	0.927
			TBUS 16	0.968	0.978	0.980
			TBUS 17	0.990	0.997	0.991
			TBUS 18	0.933	0.887	0.894

			TBUS 19	0.896	0.948	0.959
			TBUS 20	0.983	0.994	0.998
			TBUS 21	0.855	0.916	0.976
			TBUS 22	0.972	0.982	0.992
			TBUS 23	0.974	0.946	0.969
4.	**Jajpur**	9	TBUS 24	0.959	0.874	0.893
			TBUS 25	0.931	0.912	0.887
			TBUS 26	0.830	0.881	0.912
			TBUS 27	0.951	0.847	0.886
			TBUS 28	0.968	0.973	0.956
			TBUS 29	0.917	0.981	0.991
			TBUS 30	0.861	0.990	0.987
			TBUS 31	0.906	0.893	0.926
			TBUS	0.924	0.974	0.995
5.	**Mayurbhanj**	15	TBUS 33	0.988	0.952	0.925
			TBUS 34	0.977	0.991	0.884
			TBUS 35	0.859	0.997	0.997
			TBUS 36	0.985	0.989	0.991
			TBUS 37	0.970	0.990	0.976
			TBUS 38	0.893	0.992	0.994
			TBUS 39	0.835	0.921	0.928
			TBUS 40	0.978	0.957	0.891
			TBUS 41	0.964	0.884	0.887
			TBUS 42	0.912	0.829	0.912
			TBUS 43	0.989	0.857	0.986
			TBUS 44	**0.998**	0.984	0.886
			TBUS 45	0.828	0.971	0.998
			TBUS 46	0.996	**0.998**	**0.999**
			TBUS 47	0.979	0.997	0.981
6.	**Khorda**	3	TBUS 48	0.995	0.997	0.977
			TBUS 49	0.882	0.961	0.872
			TBUS 50	0.989	0.988	0.946

Maximal correlation coefficient of each places are shown in **bold**

Significant at $P < 0.01$.

Interrelationship among antigenic protein from phase II (acute) infected samples with Ab titres:

The intensification of mycobacterial antigens remains notably positive correlated adjacent to the TB serum concentration at phase II of TB infection. Positive correlation ($r = 0.998$) was found from infected samples assemble from Balasore, Mayurbhanj and Khorda districts of Odisha followed by Mayurbhanj and Bhadrak districts correlation with ($r = 0.997$). However, some other regions of Odisha obsessed lesser correlation assessment in relation with samples collected from phase III. Phase III of TB infection shows high positive correlation because these regions of Odisha are prone to TB which is transmitted or carry out by infected person only. However, TB is more common for HIV positive patients. HIV positive patient significantly got infected from TB if they are not treated or diagnosed properly. Henceforth, it was discovered that the antigenic protein act as significantly more in these regions.

Interrelationship among infected urine samples of phase III (chronic) antigenic load and Ab titres:

A positive correlation obtained among the conc. of mycobacterial proteins and TB serum from phase III of infection. However, highest positive correlation (r = 0.999) with significant value at P < 0.01 was found in samples assembled from Balasore and Mayurbhanj districts of Odisha. Similarly, low corrletion (r = 0.872) and (r = 0.874) was noticed in samples assembled from Khorda and Bhadrak districts of Odisha. Obtained of low correlation might be due to AB response in contrast to mixed infection. Similarly, chronic phase of infection the antigenic concentration is higher and the Ab-Ag complex is small, results of cross-reactivity was found with non- targeted common urine isolates microorganisms like other mycobacteria, and *Candida* because of their similar morphological to MTB (Welch et al., 1984).

Validation of Indirect ELISA assay:

The specificity and sensitivity test has been calculated from collected infected and non-infected urine samples have been estimated by using hypothetical sets of results. Indirect ELISA was performed with a sample range consisting of 100 of infected & non-infected urine samples. The symptomatic specificity and sensitivity was measured and calculated as 93.75 % and 96.15 % respectively shown in table 4. Hence, it was confirmed that the TB serum that obtained from TB patient have high capacity to detect mycobacterial antigenic protein at early phase of infection.

Table 4. Estimation and calculation of diagnostic sensitivity and specificity from hypothetical sets of result from infected and non-infected urine samples of different regions.

		No. of reference sample	
		Infected	Non Infected
Test result	Negative	50 TP	FP 3
	Positive	2 FN	TN 45
		Diagnostic sensitivity TP/(TP+FN) = 50/52 = 96.15%	Diagnostic specificity TN/(TN+FP) = 45/48 = 93.75%

IV. CONCLUSION

This research study established the effect of TB serum upon the mycobacterial antigenic protein detection. This work presented data for early and rapid detection of *Mycobacterium tuberculosis* by serological techniques from using urine samples at different phases of TB infection. Mycobacterial proteins at early phase were preferred with minimum conc. of 0.114 mg/ml having incubation period of 12-16 hr for early and rapid diagnosis by employing Indirect ELISA. We enhanced the reaction condition for TB serum in the control serum. 1: 2500 preparations with incubation period for 45 min at room temperature were selected for the parameter for control serum. (TMB: 3,3′, 5,5′- tetramethylbenzidine) substrate was employ for color outcome that confirmed enzymatic transformation at optimal density at 450 nm. All these conclusions allow us to design an effortless, fast, specific and sensitive ELISA kit which can identify mycobacterial antigenic protein at early phase of TB infection. Endemic and resource- limited settings country like India where even easy technology such as sputum smear microscopy set as poor but these serological assays will continue to serve a useful purpose. Outbreaks will be detected earlier and controlled more rapidly. Incorrect TB diagnosis based on false-positive culture results will be identifying more easily. A progress towards TB elimination can monitor more efficiently.

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