Original article

Serum adenosine deaminase activity: A novel test for early diagnosis of pulmonary tuberculosis - What about efficacy?

Abdul Rasheed Qureshi[1,2], Muhammad Irfan[3], Muhammad Sajid[4], Zeeshan Ashraf[5]

[1]Head of Department, Pulmonology OPD-Gulab Devi Teaching Hospital Lahore, Pakistan, [2]Director, Institute of Biotechnology Department, Gulab Devi Educational Complex, Lahore, [3]Pulmonologist, Gulab Devi Chest Hospital, Lahore, [4]Medical Officer, Gulab Devi Teaching Hospital, Lahore, [5]Lecturer, Department of Statistics, Gulab Devi Educational Complex, Lahore-Pakistan.

Correspondence to: Muhammad Irfan, Email: irfan16d2@gmail.com

ABSTRACT

Background: Pulmonary tuberculosis is a tremendous public health problem, increasing significantly, especially in developing countries. Getting a TB-free globe appears to be not more than a dream. Mycobacterial culturing is the gold standard for precise diagnosis but requires six-week time, in the meantime, patients remain a source of inadvertent disease dissemination in the community. The unavailability of sputum also poses a challenge several times. As adenosine deaminase level measurement is easy, rapid and independent of a sputum sample, this study was conducted to determine the diagnostic efficacy of adenosine deaminase activity for pulmonary tuberculosis.

Patients and methods: This prospective study was conducted at Pulmonology-OPD, Gulab Devi Chest Hospital, Lahore, from 01-01-2019 to 30-06-2019. A total of 300 sputum smear-positive patients with clinical and radiological evidence consistent with diagnosis of pulmonary tuberculosis and 30 normal participants without any clinical, radiological or haematological evidence of tuberculosis were included. While, smear-negative patients and those having any evidence of hepatic disease, hematopoietic malignancy, infectious mononucleosis, typhoid or pregnancy were excluded. The serum ADA level of each participant was determined. All patients were subjected to AFB culture. Diagnostic accuracy was determined by considering sputum culture as a gold standard.

Results: At cut-off value of >15 IU/L, a sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of 98.30%, 100.00%, 100.00%, 85.71%, 98.46% respectively were defined.

Conclusion: Serum ADA level, having excellent sensitivity and specificity, can diagnose pulmonary tuberculosis even if sputum sample is not available.

Keywords:

Serum, ADA level, TB-diagnosis, Sensitivity, Specificity.

INTRODUCTION

Tuberculosis (TB) remains a major public health issue of great concern globally, even in 2020. About 1/3rd of the world population is infected with *Mycobacterium tuberculosis* (MTB or *M. tuberculosis*), resulting in two million deaths and nine million new cases per year, of which 95% are found in developing countries.[1] TB remains one of the top ten causes of death worldwide.[1] It has been estimated that 562,000 HIV- negative and 3,800 HIV positive people developed tuberculosis in Pakistan in 2018.[2] MTB behaves differently in patients of AIDS, making its detection difficult in sputum samples due to pausi-bacillary attitude. According to WHO report, childhood-TB is a considerable affliction, and around 20% of cases are detected under the age of fifteen every year.[3] Furthermore, quite a significant number of patients with pulmonary shadows consistent with TB, either does not produce sputum or are unable to expectorate, resulting in non-availability of a sputum sample, making diagnosis impossible. Additionally, MTB is not detected in many expectorating patients due to low bacillary load or technical errors. Tuberculosis develops due to delayed hypersensitivity reaction in response to mycobacterial antigens.[4] The immunologic response leads to the stimulation and differentiation of lymphocytes, releasing lymphokines, but despite tremendous morbidity and mortality, TB is an easily treatable disease with anti-TB drugs. The disease can be controlled efficiently if diagnosed timely.[5] Symptoms of pulmonary TB (PTB) are usually nonspecific, while Acid Fast Bacilli (AFB) detection by culture is the gold standard for diagnosis, but this test requires at least six weeks for the result. During this time, patients' disease worsens and patients keeps on spreading infection in the community. Sputum AFB-smear is being utilized in most centres because of rapid results, but it shows false-negative results in significant numbers, and patients remain a constant source of inadvertent disease dissemination in the community.

Conflict of interest: The authors declared no conflict of interest exists.

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Sputum GeneXpert provides rapid results but implementation and maintenance of machine is costly, logistically challenging and creates a need for more affordable and durable testing platform.[6] Chest X-ray provides probable diagnosis and several times differentiation from pneumonia and malignancy become difficult. Erythrocyte sedimentation rate (ESR) is also nonspecific, may be elevated in variety of disorders. The risk of disease dissemination makes early diagnosis, a matter of utmost concern for an effective TB-control program. Due to limitations of conventional testing modalities, our mission for a TB-Free Pakistan with a vision of achieving a zero TB-death rate, for which National Strategic Plan 2017 – 2020 was designed, seems to be failing.[7]

Adenosine deaminase (ADA) is an enzyme of purine metabolism. Its level increases in serum when T-lymphocytes are stimulated by MTB and is essential for lymphocytic growth and differentiation. Chikkahonnaiah and co-authors , concluded their study that serum ADA level has a potential to be used as surrogate marker for the diagnosis of sputum smear negative and extra pulmonary TB[8] Previous studies have also supported the diagnostic usefulness of ADA in pleural tuberculosis.[9-10] Due to the limitations of conventional testing modalities, there has been a real need of a novel diagnostic test, capable of providing quick and precise TB-diagnosis, independent of sputum sample and bacillary load.[11]

As Pakistan ranks 5th among high burden countries of the world, precise diagnosis is essential for good TB-control program, it is imperative to develop a test, free from drawbacks of the conventional diagnostic tools. This study aims to determine the diagnostic efficacy of serum ADA level for pulmonary tuberculosis.

PATIENTS AND METHODS

This prospective study was conducted at Pulmonology-OPD, Gulab Devi Chest Hospital, Lahore, from 1st January 2019 to November 30, 2019. Ethical Approval no. Admin / GDEC /265/19 was obtained from IRB of the hospital and 300 patients with PUO, productive cough for >03 weeks, symptoms, signs and radiological features consistent with pulmonary tuberculosis and sputum positive for AFB smear by fluorescent microscopy (F.M.) were included and randomly distributed. Thirty normal healthy participants, not having any TB-patient in their family, not having any fever, cough, sputum and normal CXR, CBC and ESR, were included as control. Smear negative patients, drop-out cases, patients having hepatic disease, bronchogenic carcinoma, any known history of hematopoietic malignancy, infectious mononucleosis, typhoid, pregnancy, and those not willing for participation were excluded. After obtaining an informed consent, 3 ml venous blood was drawn from each patient and control, aseptically on day zero. Serum was extracted by centrifugation and was subjected to ADA estimation by colorimetric method. Serum ADA activity was expressed as IU/L (international unit per liter). All patients were subjected to AFB-culture on Lowenstein Jensen (L.J.) medium. The healthy control and patients were evaluated with CBC, ESR and TB-patients were additionally investigated by LFTs, viral markers and Widal Test. ADA value of >15 IU/L was considered as cut-off for TB-diagnosis. Clinical diagnosis was made by correlating history, physical and radiological findings along with blood reports and sputum AFB smear and culture. GeneXpert was also utilized in pertinent cases. Anti-TB treatment was initiated and completed according to DOTS protocol.

SPSS-16, software was used for data entry and analysis. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were calculated by considering sputum AFB culture as reference. Mean ADA value in TB-patients and control were computed. A p-value <0.05 was taken as statistically significant.

RESULTS

Among total 330 subjects, 300 with pulmonary tuberculosis (PTB) and 30 controls, participated in the study. Out of 330 patients, 173 (52.42%) were male while 157 (47.58%) were female. Male to Female ratio was 1.10:1. Age range was 15-73 years with mean age was 30.67 $\pm$ 13.50 years. Patients presented with classical lower respiratory complaints (Table 1).

Total 300 patients were diagnosed as cases of TB by history, physical, radiological and hematological examinations along with sputum AFB smear reports. Sputum AFB culture on L.J. medium proved TB in 294 patients, 04 cases were diagnosed as atypical

Table 1. Frequency of clinical features in 300 patients with PTB

History Findings	Observed cases	Percentage
Cough	300	100.00%
Sputum	300	100.00%
Fever	279	93.00%
Night sweats	270	90.00%
Decreased Appetite	259	86.33%
Weight loss	182	60.66%
Hemoptysis	48	16.00%
Shortness of Breath	96	32.00%
Contact History	52	17.33%

mycobacterium while 02 cases showed no growth and were later diagnosed as organizing pneumonia. Serum ADA level was performed on 294 TB-patients and 30 control subjects. It ranged from 05 IU/L to 82 IU/L for TB-patients. Cut-Off value of >15 IU/L was considered for TB-diagnosis. Patients with ADA level >15 IU/L were 289/294 (98.29%) while 05/294 TB cases (1.70%) exhibited ADA level < 15 IU/L. Mean ADA level, in TB cases was 31.29 IU/L, SD (±13.82). Thirty control participants showed a range from 05 to 09 IU/L. Mean value was 7.95 IU/L with S.D. ± 3.17 IU/L. The Fisher exact test statistic value was 0.0471. By considering sputum AFB culture as gold standard, for calculating the efficacy of serum ADA level, total True positive cases (T.P.): 289, true negative cases (T.N.): 30, false negative cases (F.N.) : 05 and false positive cases (F.P.): 00 were identified. Computed efficacy is tabulated (Table 2).

DISCUSSION

Despite all advancement in TB-diagnosis, it is still a major public health problem & lack of timely diagnosis is a major factor for TB-control program inadequacy. Although WHO has recommended rapid molecular detection by GeneXpert but availability and cost constrain are in the way.[12,13] This study showed mean age 30.67 years while other studies reported mean age 37.6 to 49 years.[14-16] All patients (300) were already diagnosed TB on sputum AFB-smear, culture, radiological and clinical scenario. The mean ADA value for TB cases was 31.29±13.82 IU/L, while for control group it was 8.95± 3.17 IU/L. None of the control-participants could reach the cut-off point (p=0.047). It revealed that there is much difference in ADA level between TB-patients and normal healthy control, enabling rapid differentiation between a healthy person and a TB-patient. The highest level of serum ADA was noted in young and patients with advance disease on x-ray chest or with higher grade of sputum positivity. ADA activity may depend upon the pathologic stimulus like bacillary load and rapidity of T-lymphocyte proliferation. Five patients in this study had ADA level, less than 15 IU/L, out of which two patients were young (35-38 years) with minimal lesion in upper lobe

Table 2. Efficacy of serum adenosine deaminase level in diagnosis of pulmonary TB

Statistic	Value %	95% confidence interval
Sensitivity	98.30%	96.08% to 99.45%
Specificity	100.00%	88.43% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	85.71%	71.56% to 93.47%
Diagnostic accuracy	98.46%	96.44% to 99.50%

That is why in-spite of being sputum positive, lower ADA level was obtained, remaining three patients, belonged to elderly age group (65-70 years). The biologic plausibility for negative correlation between ADA and age is due to ageing, which is associated with remodeling of the immune system, leading to decline in immune efficacy, resulting in increased vulnerability to infectious diseases. An age-related immune alteration includes the reduction in the number of peripheral blood naïve cells and relative increase in the frequency of memory cells. Therefore, there is a need for interpretation of ADA level according to patient age and the quantity of disease on x-ray chest. So a lower cut-off value should be used for elderly people and those having minimal disease on CXR. In this way, the number of false negative results can be minimized, and treatment can be instituted early. Shahla and co-authors reported average ADA values in TB and non-TB patients, 20.88 (±5.97) and 10.69 (±2.98) IU/L, respectively at cut-off point 14 IU/L.[17] Two other studies revealed markedly elevated ADA level in TB-patients as compared to healthy individual.[18,19] Lakshmi and colleagues reported serum ADA levels in TB-patients 33.52±15.22 IU/L while healthy controls had 16.5±3.18 IU/L. Thakur and coworkers reported ADA levels in TB-patients and healthy subjects as 56.7±14.43 IU/L and 22.9±3.87 IU/L, respectively.[20] These studies described their results as statistically significant (p<0.05), which is in agreement with findings in this study.

On the other hands, Fernandez and co-workers commented that there was no difference of ADA level in TB-patients and healthy individual. Dinnes and colleagues pointed out that ADA level is not diagnostic for differentiating TB from other pulmonary infections.[21] The debate about the ability of ADA, to differentiate between pulmonary TB and non-TB infections continues since many years. Alaarag and colleagues reported that Serum ADA level shows higher percentage positivity compared with clinical, radiological, and laboratory parameters in the diagnosis of pulmonary TB Serum ADA level increases in patients with all forms of TB as compared to non-tuberculous lung diseases such as pneumonia, bronchiectasis, lung abscess, and lung cancer or normal individuals.[22] Joshaghani and coworkers stated that ADA estimation in serum can be useful for differentiation of healthy subjects from respiratory disease. Collazos et al described elevated ADA level in TB-patients and a significant decline by two months treatment with a p-value <0.05. Yurt and coworkers concluded that ADA

estimation may find a place as routine investigation in future for the diagnosis of tuberculosis.[23] Similarly Dilmaç and colleagues revealed higher values for TB than cancer & COPD with P < 0.01 for lung cancer and p< 0.05 for COPD. Taparia and co-authors reported the sensitivity, specificity, PPV, and NPV, 93%, 80%, 93.3%, and 80%.[24] Kanchan and associates described sensitivity of 93.93%, specificity of 96.69%, PPV of 96.87%, and NPV of 94.11% while Kuyucu and group revealed a sensitivity of 100%, specificity of 90.7%, PPV of 58.8%, and NPV of 100%.[25] This study found sensitivity, specificity, PPV NPV & diagnostic accuracy as 98.30%, 100.00%, 100.00%, 85.71%, 98.46% respectively, which is in agreement with the above mentioned studies.

According to WHO report, about 20% TB-patients belonging to pediatric age group is usually a real challenge.[26] Mishra and coworkers reported significantly increased ADA levels in tubercular children than healthy, with a p-value of <0.001.[27] Similarly, Yasuhara and coauthors concluded that serum ADA activity in pediatric patients with active PTB was significantly greater than those with bacterial or viral pneumonia.[28]

Main limitation of the study is that this is a single centered, first study in Pakistan on this subject. By increasing the sample size and preferably involving multiple centers, the subject can be more confidently explored. In future studies, instead of recruiting normal healthy individuals as control, patients with common pulmonary infections should be included as control, in the same sets of conditions, for increasing the power of the study. Because BCG vaccinated children show elevated levels of ADA without TB disease, a study is really needed for defining an independent cut-off value for diagnosing TB in BCG vaccinated children below 10 years of age.

This study revealed that serum ADA level with 100% specificity can be a useful alternative test to rule out the diagnosis of PTB. So it can be used to differentiate between TB and non-TB patients. Similarly, 98.30% sensitivity highlights, a good ruling in capacity of the test. So it can be commented that this test may be employed for the diagnosis of pulmonary TB even in problematic patients like symptomatic false negative (smear), milliary shadows, dry cough, weak, unconscious or non-cooperative and HIV-positive patients. Children can also be worked up by keeping an eye on the effect of BCG. It is very pertinent to make a note here that this test is rapid, report can be available within one hour, cuts down the time for investigation, can prevent inadvertent disease dissemination in community, and can strengthen the TB-control program.

CONCLUSION

Serum ADA level estimation is rapid, easy, reliable and specific tool for diagnosing Pulmonary Tuberculosis. It may help in early administration of specific Anti-TB drugs and might be very useful for TB-control program, by cutting down the chain of disease transmission. It is capable of diagnosing difficult TB cases where sputum is not available/non-conclusive, HIV-TB cases or extra-pulmonary TB

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REFERENCES

1. GBD Tuberculosis Collaborators. The global burden of tuberculosis: results from the Global Burden of Disease Study 2015. Lancet Infect Dis. 2018;18(3):261-284.
2. Global TB Report, WHO, 2019. [Internet], 2019[cited 2019 July 20] Available from:https://www.who.int/tb/publications/global_report/en/
3. Subregional workshop on childhood tuberculosis (TB), 2013.[Cited 2013 Augurst 8]Available from: http://www.emro.who.int/pak/pakistan-events/sub-regional-workshop-on-childhood-tb.html
4. Lende TG, Waghmare P, Ambilkar AW, Kumar S. Predictive value of serum adenosine deaminase levels in prospect of tubercular infections. BBRJ. 2019;3(2):105.
5. Oxlade O, Schwartzman K, Behr MA. Global tuberculosis trends: A reflection of changes in tuberculosis control or in population health? Int J Tuberc Lung Dis. 2009;13:1238–1246.
6. Cowan J, Michel C, Manhiça I, Monivo C, Saize D, Creswell J, et al. Implementing rapid testing for tuberculosis in Mozambique. Bull World Health Organ. 2015;93(2):125-30.
7. National END TB Strategic Plan 2017-20, Available from: https://secureservercdn.net/104.238.71.109/9aa.913.myftpupload.com/wp-content/uploads/2020/07/nets.pdf
8. Chikkahonnaiah P, Jaggi S, Goyal B, Garg K, Gupta S, Jaswal S, et al. Utility of serum ADA estimation in the diagnosis of extrapulmonary tuberculosis. J Med Sci Clin Res. 2017; 5(5):21549-53.
9. Mchedlishvili N, Mamaladze T, Shubladze N, Gujabidze N, Marashanishvili R. Diagnostic value of pleural fluid adenosine deaminase level in patient with tuberculous pleurisy. Eur Respiratory Soc; 2016; 48: PA2768.
10. Aggarwal AN, Agarwal R, Sehgal IS, Dhooria S. Adenosine deaminase for diagnosis of tuberculous pleural effusion: A systematic review and meta-analysis. PLoS One. 2019 26;14(3):e0213728.
11. Mishra OP, Yusaf S, Ali Z, Nath G, Das BK. Adenosine deaminase activity and lysosyme levels in children with tuberculosis. J Tropical Pediatr. 2013;46:175–178.
12. Jobayer M, Shamsuzzaman SM, Zulfiquer K. Rapid Diagnosis of Pulmonary Tuberculosis From Sputum by Polymerase Chain Reaction. Arch Clin Infect Dis. 2014;9(2) e20694.

13. Cowan J, Michel C, Manhiça I, Monivo C, Saize D, Creswell J, Gloyd S, Micek M. Implementing rapid testing for tuberculosis in Mozambique. BBRJ. 2014;93:125-30.
14. Alaarag AH, Mohammad OI, Farag NM. Diagnostic utility of serum adenosine deaminase level in the diagnosis of pulmonary tuberculosis. Egypt J Bronchol. 2016;10(2):133-9.
15. Salmanzadeh S, Tavakkol H, Bavieh K, Alavi SM. Diagnostic value of serum adenosine deaminase (ADA) level for pulmonary tuberculosis. Jundishapur J Microbiol: 2015;8(3).
16. Lende TG, Waghmare P, Ambilkar AW, Kumar S. Predictive value Of serum Adenosine Deaminase levels In prospect of tubercular infections. BBRJ. 2019;3(2):105.
17. Afrasiabian S, Mohsenpour B, Bagheri KH, Sigari N, Aftabi K. Diagnostic value of serum adenosine deaminase level in pulmonary tuberculosis. J Res Med Sci. 2013;18(3):252.
18. Badade ZG, Narshetty GS, Shah VK, Potdar PV, More K, Badade VZ. Study of serum adenosine deaminase (ADA) level in diagnosis of extrapulmonary and smear negative tuberculosis. Biochemistry. 2015;4(12).
19. Boloursaz M, Khalilzadeh S, Khodayari A, Hakimi S. Adenosine deaminase level as an indicator for differentiating between active pulmonary tuberculosis infection and other pulmonary infections. J Compr Pediatr. 2012;3(1):3-6.
20. Gajwani T, Ahuja J. A comparative study of serum adenosine deaminase enzyme and serum peroxidase albumin ratio in diagnosis of pulmonary tuberculosis. Int J Curr Res Rev. 2012;4:53-8.
21. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technology Assessment (Winchester, England). 2007;11(3):1-96.
22. Alaarag AH, Mohammad OI, Farag NM. Diagnostic utility of serum adenosine deaminase level in the diagnosis of pulmonary tuberculosis. Egyptian J Bronchol. 2016;10(2):133-9.
23. Yurt S, Küçükergin C, Yigitbas BA, Seçkin Ş Tigin HC, Koşar AF. Diagnostic utility of serum and pleural levels of adenosine deaminase 1–2, and interferon-γ in the diagnosis of pleural tuberculosis. Multidiscip Respir Med. 2014;9(1):1-7.
24. Taparia P, Yadav D, Koolwal S, Mishra S. Study of lipid profile in pulmonary tuberculosis patients and relapse cases in relation with disease severity-A pilot study. Indian J Sci Appl Res. 2015;2:41-50.
25. Kanchan S, Varma Santosh G, Sawale Vishal M, Abhichandani Leela G, Niyogi NG. Joshi A. Study of adenosine deaminase levels in patients of pulmonary TB with and without pleural effusion. IOSR J Dent Med Sci. 2014;13:30-7
26. National TB Control Programme [Internet], 2019[cited 2019 July 20] Available from: https://ntp.gov.pk/national-tb-data/
27. Mishra OP, Yusaf S, Ali Z, Nath G, Das BK. Brief report. Adenosine deaminase activity and lysozyme levels in children with tuberculosis. J. Trop. Pediatr. 2000;46(3):175-8.
28. Yasuhara A, Nakamura M, Shuto H, Kobayashi Y. Serum adenosine deaminase activity in the differentiation of respiratory diseases in children. Clinica Chimica Acta. 1986;161(3):341-5.
29.

