A STUDY OF SERUM ADA LEVELS AND ITS PROGNOSTIC VALUE IN CHILDHOOD TUBERCULOSIS

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HOW TO CITE THIS ARTICLE:

A. Amaresh, J. N. George, T. Karthik Bharadwaj. "A Study of Serum ADA Levels and its Prognostic Value in Childhood Tuberculosis". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 22, June 02; Page: 6119-6125, DOI: 10.14260/jemds/2014/2711

ABSTRACT: BACKGROUND: Tuberculosis is one of the most widely prevalent infectious disease causing high morbidity and mortality in humans. Serum ADA is a low cost test with proven diagnostic significance. **AIMS AND OBJECTIVES:** To assess change of Serum ADA levels as an indicator of response to the treatment, the prognostic value of ADA levels in childhood tuberculosis and its role in early detection of drug resistance or treatment failure if any. **MATERIALS AND METHODS:** A prospective analytical study (n=59) was conducted in Niloufer hospital from July 2012 to September 2013. A total of 32 patients were enrolled in tuberculous meningitis group and 27 in pulmonary tuberculosis group. Blood samples were drawn at the time of diagnosis, 1 month and 2 months after starting treatment. ADA levels were measured using Guisti's method. Data collected was analyzed using SPSS 16 software. **RESULTS:** Mean Serum ADA levels on Day 1, 1 month, 2nd month samples were [46.97±11.00], [31.51± 6.27], [21.67± 4.00] and [32.31± 5.90], [24.03± 4.38], [18.43± 2.76] in Pulmonary Koch's and TBM group respectively. Serum ADA levels showed a significant decrease (p < 0.000) after the initiation of treatment. Sex, age and nutritional status as measured by weight for age did not have a significant effect on Serum ADA value. There was a significant difference between mean ADA levels in the pulmonary tuberculosis group and TBM group but age was confounding factor. In the TBM group, 5 children who succumbed to the disease had a significantly (p = 0.016) lower Serum ADA levels (mean = 22.08) when compared to other children who have not succumbed to the disease. The relative risk of a TBM child dying with the disease was calculated to be 8.9 if the Serum ADA levels were less than 22IU/dl. **CONCLUSIONS:** There was a significant sequential decrease in Serum ADA levels in tuberculosis patients with treatment and these were not influenced by age, sex and nutritional status. A low level of Serum ADA in TBM is a significant negative prognostic indicator.

KEYWORDS: Tuberculosis, Serology ADA, Prognosis.

INTRODUCTION: Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. Approximately 8-10million people are infected with TB every year.¹ In the year 2011, 8.7million people fell ill with TB and 1.4million died from TB. World Health Organization (WHO) estimates in 2012 revealed that, up to 74,000 children die from TB each year and children account for around half a million new cases annually.²

Since most children acquire the organism from adults in their surroundings, the epidemiology of childhood tuberculosis follows that in adults. Due to difficulty in confirming the diagnosis, the global burden of childhood tuberculosis in the world is unclear and often under estimated. Several estimates make use of an arbitrary calculation assigning 10percent of the tuberculosis burden to children.³ Childhood TB may account for up to 20-40% of the cases in high burden countries like India.⁴

ORIGINAL ARTICLE

Childhood TB remains a hidden epidemic in most countries.⁵ Multi drug resistant (MDR) TB is one of the major challenges the world is facing and is estimated to be about 15% of all tuberculosis cases in our country. Data on childhood MDRTB in India is very scarce. A study at AIIMS showed that out of 1579 children registered over a period of 8 years, 21 [1.32%] had MDR TB.^{6,7} Over the years though the incidence of TB has increased, it has been largely due to increase in the population.⁸

With successful implementation of DOTS therapy, world over the mortality due to TB has been reduced to a great extent. Early identification of active TB is crucial to combat the TB epidemic.^{9,10} Identification of latent TB, especially in high risk groups, assessment of disease activity, response to treatment and early identification of relapse would be very useful. Some studies have indicated that shorter courses of therapy may be possible in early responders, thereby increasing the compliance and treatment outcome.

Currently one of the most widely used biomarker for TB is serum Adenosine deaminase (ADA), an enzyme involved in purine metabolism. ADA catalyzes hydrolytic cleavage of adenosine, converting it to nucleoside inosine and ammonia. There are two isozymes – ADA 1 & 2. ADA 1 is ubiquitous while ADA 2 is mostly found along with ADA1 in macrophages and lymphocytes. ADA acts in proliferation and differentiation of lymphocyte and especially T lymphocyte. It acts in maturation of monocytes and transforming them to macrophage.

It is a significant indicator of active cellular immunity. The level of serum ADA increases in various diseases in which cell mediated immunity is stimulated. Mycobacterium tuberculosis evokes a strong T cell mediated response which probably reflects the raised ADA levels in various body fluids in tuberculosis.^{11,12} ADA levels have been found to be extremely useful in diagnosis of tuberculosis over the years. Previous studies have confirmed the diagnostic value of ADA activity in effusions due to pleural, pericardial, meningeal and peritoneal.

MATERIALS AND METHODOLOGY: This is a Prospective analytical study conducted at Niloufer hospital, a tertiary care pediatric teaching hospital affiliated to Osmania medical college, Hyderabad. The study was conducted from July 2012 to September 2013 and is approved by Institutional Ethics Committee. All new cases of tuberculosis in children between 1 and 15 years of age are included in the study. The diagnostic criteria are isolation of organism through culture, Positive sputum smears, Positive Mycobacterial TB, PCR from body fluids, characteristic histopathological findings in biopsies, Characteristic cytological, biochemical and ADA levels in body fluids, Other supportive evidences like Montoux, Quantiferon gold, ESR, Characteristic radiological features and finally, decision by the physician to treat the case as tuberculosis.

Exclusion criteria are co-existence of diseases like Bronchiectasis, Empyema, Lung abscess, Malignancies, SLE, Rheumatoid arthritis, Psoriasis which are known to raise ADA levels and cases of tuberculosis in whom treatment has been already initiated. The Sample size is 59 patients, and Informed Assent/Consent was taken from patient / parent or guardian. History, physical examination done and reviewed the available lab data. 2 ml of blood was collected in a plain container and processed within 2 hours of collection.

Blood for Serum ADA levels was collected on days 1, after 1 month and 2 months and was sent for biochemical analysis. Appropriate physical examination and history was collected during follow up dates. The child was observed for the above period and death as final outcome was

ORIGINAL ARTICLE

measured. Data collected was entered in Microsoft Excel spread sheet and was analyzed using SPSS 16 software using Fixed Effects Model.

OBSERVATION AND RESULTS: Out of the 59 patients, 32 belonged to TBM/ disseminated Koch's group and 27 to pulmonary Koch's group. 3 patients were lost to follow up in each group. Serum ADA levels were measured in each group and they showed a very significant decrease ($p < 0.000$) after the initiation of treatment. Results of the Pulmonary TB group were similar but age did not have a significant effect on intra group ADA levels. The mean age of the subjects was 9 years.

The mean values of Serum ADA were $[46.97 \pm 11.00]$, $[31.51 \pm 6.27]$ and $[21.67 \pm 4.00]$ at diagnosis, end of 1st month and 2nd month respectively. Regression analysis showed that sex and age did not have any significant effect on the ADA levels. Weight for age Percentiles were calculated using Agarwal et.al,¹³ Affluent Indian growth charts. ADA levels were not influenced by the nutritional status of the child.

TB meningitis & disseminated Koch's group showed interesting results. Mean age of the subjects was 5 years and 7 months. Serum ADA levels significantly decreased with DOTS therapy.¹⁴ The mean values of Serum ADA were $[32.31 \pm 5.90]$, $[24.03 \pm 4.38]$ and $[18.43 \pm 2.67]$ at diagnosis, end of 1st month and 2nd month respectively. Five children who were enrolled in the study, died within 1 week to 10 days after the diagnosis. They showed a significantly lower ADA levels (mean – 22.08) than those who survived ($p = 0.016$).¹⁵ Age, sex and nutritional status did not have a significant effect on ADA levels. The relative risk of a child with TBM dying with the disease was calculated to be 8.9 if the serum ADA level was below 22 IU/dl.

DISCUSSION: Our study showed that there was a significant sequential decrease in serum ADA levels in patients with tuberculosis. There are many studies which have evaluated the diagnostic significance of Serum ADA, but none in evaluating it as a prognostic indicator.

The mean values of Serum ADA were $[46.97 \pm 11.00]$, $[31.51 \pm 6.27]$, $[21.67 \pm 4.00]$ and $[32.31 \pm 5.90]$, $[24.03 \pm 4.38]$, $[18.43 \pm 2.67]$ at diagnosis, 1st month and 2nd month in pulmonary tuberculosis and tuberculous meningitis respectively.¹⁶ The values were lower than what have been found in other studies. Differences in age group and laboratory technique may explain the variation.

The mean Serum ADA¹⁷ levels in tuberculous meningitis were significantly lower than those observed in pulmonary tuberculosis. Age with correlation co-efficient of 0.5 had a partial positive correlation and its confounding effect on the above finding could not be excluded. Another finding in our study is that a lower level of Serum ADA levels in tuberculous meningitis¹⁸ had a grave prognostic value. A relative risk of 8.9 was obtained when a cut off value of 22.01 IU/dl was taken.

The above findings may be explained from the fact that younger children mount a different immune response to tuberculous pathogen when compared to adults. The relative anergy to tuberculous pathogen in young children makes them prone for severe forms of tuberculosis with higher mortality and morbidity.

Low levels of Serum ADA in children who have died may possibly be a reflection of this anergy though larger and more controlled studies are required to validate this fact. A recently published study by Filiz Cimen et al, on serum ADA levels in pulmonary TB in adults which included drug resistant TB, TB relapse cases along with other WHO categories of tuberculosis showed that ADA levels were lower in relapse cases and also drug resistant cases when compared with other

ORIGINAL ARTICLE

groups. They also further state that ADA values became lower and lower as resistance to number of drugs in the organism has increased.¹⁹

Thus it might not be inappropriate to infer that serum ADA has an inverse correlation with prognostication of the disease. Larger multi centric studies are required to validate the findings of our study in different populations and with different laboratory techniques.

We are of the opinion that a prognostic scoring system which may include variables like age, nutritional status, Serum ADA, clinical diagnosis at the time of presentation, etc may be a very useful tool in the clinicians hand to prognosticate Tuberculosis.²⁰ Serum ADA at a cost of less than Rs 10 per test, thus is a very important biomarker with both diagnostic and prognostic implications in Tuberculosis.

CONCLUSION: Serum ADA levels showed significant decrease with treatment in both the groups. Age, sex and nutritional status did not have a significant effect on intra group variation of Serum ADA. Five children who were enrolled in the study who died showed a significantly lower ADA levels (mean – 22.08) than those who survived ($p=0.016$).

The relative risk of a child with TBM dying with the disease was calculated to be 8.9 if the serum ADA level was below 22 IU/dl. Serum ADA was significantly ($p=0.002$) lower in TBM group when compared to pulmonary TB group although age was a confounding factor. The relative anergy towards *M. tuberculosis* as reflected by lower serum ADA levels may play a decisive role in dissemination and also the outcome of the disease.

Limitations of the Study: Single centre study with a limited cohort and other inflammatory markers were not studied and compared with Serum ADA. Definitive objective criteria like bacteriological identification by microscopy or culture were not the sole criteria for inclusion in to the study.

RECOMMENDATIONS: Larger multi centre trials should be done to further validate the results of the study. Need research to develop a scoring system for prognostication of tuberculosis in children.

Similar study including drug resistant cases to see if there is any role of serum ADA in early detection of drug resistance and also in HIV-TB. Search for cheaper and reliable biomarkers in TB for detecting drug resistant or high risk cases.

BIBLIOGRAPHY:

1. World Health Organization. The World Health Report: making a difference. 1999: 110.
2. World Health Organization: Global tuberculosis report. 2012.
3. Some current concepts on childhood tuberculosis. S.K. Kabra, Rakesh Lodha & V. Seth, Indian J Med Res 120, October 2004, pp 387-397
4. Childhood tuberculosis: out of control? Donald PR. Curr Opin Pulm Med. 2002 May;8(3):178-82
5. WHO News release 2012 Geneva
6. Essentials of Tuberculosis in Children, Vimelesh Seth, SK Kabra
7. World Health Organization: Global tuberculosis report. 2013
8. Ananthnarayan R, Jayaram Panikar CK. Text book of Microbiology. 7th edition. Universities press; 2005:351- 364
9. Epidemiological basis of TB control. In Davies PDO, clinical tuberculosis, Enarson DA, Rouillon A

ORIGINAL ARTICLE

10. Vynnycky E, Fine PE. Life time risks, incubation period and serial interval of tuberculosis. Am j Epedemiol: 152: 247-63.
11. Seth, Kabra Immunopathogenesis in tuberculosis.
12. Ahmed et. al., Chidhood Tb, A review of diagnosis and management.
13. Agarwal MK, Jitendra Nath, Mukerji PK, V.M.LSrivastava. A study of serum Adenosine Deaminase activity in Sputum Negtive patients of Pulmonary Tuberculosis. Ind J.Tub. 1991; 38: 139-141.
14. WHO 2008b. Joint TDR/expert committee on biomarkers in TB, Geneva.
15. Mishra OP, Yusaf S, Ali Z, Nath G, Das BK. Brief report. Adenosine deaminase activity and lysozyme levels in children with tuberculosis.Â J Trop Pediatr. 2000 Jun; 46 (3):175-178. [PubMed] [doi:10.1093/tropej/46.3.175]
16. Sequential Evaluation of Serum Adenosine Deaminase in Patients treated for Tuberculosis. Julio Collazos, MD; Pedro Espana, MD; Jose Mayo, M
17. Adenosine deaminase in childhood pulmonary tuberculosis: diagnostic value in serum. Kuyucu N, Karakurt C, Bilaloğlu E, Karacan C, Teziç T. journal of tropical medicine 1999(august [4]-241
18. Significance of Adenosine deaminase activity in serum in extra pulmonary tuberculosis. Stevanovic G., Pelemis M., Pavlovic M., Lavadinovic L., Dakic Z., Milosevic I., Milosevic B. Journal of Imab 2011.
19. Cimen F, Ciftci TU, Berktaş BM, Sipit T, Hoca NT, Dulkar G. The relationship between serum adenosine deaminase levels in lung tuberculosis along with drug resistance and the category of tuberculosis. Turkish Respir J 2008;9: 20-3.
20. Srinivasa RK, Kumar HA, Rudresh BM, Srinivas T, Bhat KH. A comparative study and evaluation of serum adenosine deaminase activity in the diagnosis of pulmonary tuberculosis. Biomed Res. 2010 (2): 189-94.

Distribution of Subjects: A total of 59 subjects participated in the study. Out of the 59 patients 32 belonged to TBM/ disseminated Koch's group and 27 to pulmonary Koch's group.

ORIGINAL ARTICLE

3 patients were lost to follow up in each group. Serum ADA levels were measured in each group and found significant decrease ($p < 0.000$) after the initiation of treatment.

Type III Tests of Fixed Effects^a

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	71.440	113.602	.000
Time	2	37.351	28.842	.000

a. Dependent Variable: Serum_ADA.

ORIGINAL ARTICLE

Estimates of Fixed Effects^b

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	46.973913	4.828672	62.890	9.728	.000	37.324250	56.623576
[Time=1.00]	-25.300000	3.446408	40.349	-7.341	.000	-32.263573	-18.336427
[Time=2.00]	-15.456522	2.434132	29.461	-6.350	.000	-20.431501	-10.481543
[Time=3.00]	0 ^a	0	.	.	.	.	.

a. This parameter is set to zero because it is redundant.

b. Dependent Variable: Serum_ADA.

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Date of Submission: 15/05/2014.
Date of Peer Review: 16/05/2014.
Date of Acceptance: 23/05/2014.
Date of Publishing: 31/05/2014.