Egyptian Journal of Bronchology

Vol. 4, No. 1, June, 2010

ORIGINAL ARTICLE

ROLE OF ADENOSINE DEAMINASE (ADA) IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

By

Khalid Hassanein,[1] Hossam Hosny,[1] Randa Mohamed,[2] Wagdy Abd El- Moneim[3]

[1]Chest Department, Faculty of medicine, Cairo University, [2]Chemical pathology department, Faculty of medicine, Cairo University, [3]General Directorate for chest diseases, Ministry of health, Egypt

Correspondence to: Wagdy Abd El- Moneim Amin, Email: drwagdy@yahoo.com

Background: ***Tuberculosis is still one of the major health problems in the world with more than 9 million new cases and 3 million deaths each year. Rapid and accurate diagnosis of symptomatic patients is a cornerstone of global tuberculosis normal strategies. Deficiencies in current case-finding tools in disease endemic countries have made it difficult to ensure access to good diagnostics at all health service levels, leaving many patients undiagnosed.***

Objective: ***a case control study aimed to assess the role of adenosine deaminase level in serum and Broncho Alveolar Lavage (BAL) in the diagnosis of pulmonary tuberculosis.***

Methods: ***Samples of serum and bronchoalveolar Lavage (BAL) were taken from 50 Egyptian patients to assess the level of Adenosine deaminase (ADA) in serum & broncho alveolar lavage fluid (BALF).***

Results: ***The results of this study showed that patients with pulmonary tuberculosis had significantly higher ADA level in serum and BALF than patients with non- tuberculosis lung diseases (P< 0.001) with cut off point obtained, in serum ADA level was 26.2 u/l, with sensitivity and specificity were 95% and 83.3% respectively and positive predictive value was 79.2%. In BALF ADA level, the cut off point was 2.5 u/l, with sensitivity and specificity were 100% and 83.3% respectively and positive predictive value was 80%.***

Conclusion: ***Adenosine deaminase (ADA) in pulmonary tuberculosis patients revealed significantly increased level in both serum and BALF for all patients with pulmonary tuberculosis compared to ADA level of cancer, pneumonia and normal persons.***

Keywords: ***Tuberculosis, ADA, Serum, BAL.***

INTRODUCTION

Tuberculosis continues to be a major cause of morbidity and mortality worldwide. The diagnosis is usually based on clinical presentation, radiologic findings and positive tuberculin and/or BCG tests. However, clinic-radiological features are variable and the latter tests may be falsely negative. Under such circumstances, anti-tuberculous therapy is started empirically. It therefore becomes imperative to find some rapid and useful tests for the diagnosis of tuberculosis.(1)

Egypt has intermediate level of incidence and mortality, the size of burden was a matter of concern, particularly in view of the fact that 74% of TB cases occur among socially and economically productive age groups of 15 to 54 years. The current estimate is calculated with several

epidemiological methods combined including tuberculin survey in 1997, mathematically calculation 2003 and finally nation wide re-estimation of TB burden in 2007 using new technique in data analysis (capture - recapture). The latest surveillance data in 2009 revealed that: a total of 18 000 tuberculosis cases existed in the /country with a prevalence rate of 24/100 000 population, Estimated incidence rate of 19 and 8/100,000 for all forms and smear positive tuberculosis, respectively (Unpublished Egyptian National TB Program "NTP" 2009 report)

Adenosine deaminase (ADA) is an enzyme that catalyzes the hydrolytic and irreversible deamination of adenosine to inosine as well as deoxyadenosine to deoxyinosine. The determination of ADA concentration in pleural fluid is currently used for the diagnosis of tuberculous pleurisy. There are also studies showing high levels of ADA in serum and bronchoalveolar lavage (BAL) of patients with pulmonary tuberculosis.[2,3]

Adenosine deaminase (ADA) is involved in the propagation and differentiation of various lymphocytes, particularly T-lymphocytes, so that estimation of its level of activity in various body fluids has been suggested for the diagnosis of tuberculous effusions especially pleural forms.[3,4]

Aim of the Work: This study was aimed at assess the role of adenosine deaminase (ADA) level in serum and Broncho Alveolar Lavage (BAL) for diagnosis of adult pulmonary tuberculosis.

PATIENTS AND METHOD

Study design: a case control study

Subjects: the study was conducted on fifty Egyptian patients, admitted to El-Giza chest hospital and chest department, Cairo University during period between October 2004 and March 2005. Exclusion criteria included: (Patients with Diabetes Mellitus- Immunocompromised patients - Patients on corticosteroids - Patients with hepatic and renal impairment).

Study groups were classified into (4) groups:

Group (1): (20) Patients with sputum (+ve) for AFB.

Group (2): (10) Patients with bronchogenic carcinoma, diagnosed clinically, radiologically & histo-pathologically.

Group (3): (10) Patients with pneumonia, diagnosed clinically, radiologically and bacteriologically.

Group (4): (10) Normal healthy persons.

Methods:

ALL Patients were subjected to the following:

1) Thorough history taking and clinical examinations.
2) Plain chest x-ray (postero-anterior & lateral view).
3) Sputum for AFB.
4) Routine laboratory investigations including: CBC & Differential count, Liver & Renal Function Tests.
5) ADA in serum & BAL fluid.
6) CT- Scan in selected cases.

Collection of sputum specimens: Early morning sputum sample was obtained from each patient on 3 successive days. Patients were instructed to take deep breath, hold it momentarily then cough vigorously into sterile screw-capped 50 ml plastic containers.

Broncho alveolar lavage technique: The technique is accomplished by wedging the tip of the bronchoscope into a segmental bronchus which harbors the abnormal shadows in the x-ray (for the case) and into the middle lobe of the lingula (for the control). Other lobes can be used, but lavage of the upper lobe is more difficult because of the tight hend in the bronchoscope imposed by the anatomy of the upper lobe bronchi,[5] once the bronchoscope was wedged, 5 sequential aliquots of 20 ml 0.9% sterile saline are injected into the channel part. The fluid is immediately pulled back with gentle suction following each aliquot. The lavage fluid was pooled in a sterile bottle.

The enzyme activity level was measured by Giusti method.[6] Data analysis was performed by Chi-Square, ANOVA and LSD tests. The significant level was evaluated for P value of less than 0.05.

Statistical analysis: A data entry sheet on Microsoft Excel software was used to the data entry, SPSS v.16 was used to statistically analyze the data, student's t-test used for comparison between tuberculosis and lung cancer patients. Statistical significance was set at p=0.05. Receiver operating characteristics (ROC) curves were constructed in order to establish a sensitivity specificity relationship for best cut off values.

RESULTS

Out of patients selected, 14 males and 6 females were diagnosed as pulmonary tuberculosis, 8 males and 2 females were diagnosed as bronchogenic carcinoma, 8 males and 2 females were diagnosed as pneumonia, 8 males and 2 females were normal group. 38 (76%) patients were males and 12 (24%) were females.

Table 1. Age of patients in different groups.

	No.	Mean	SD	Minimum	Maximum
Pul TB	20	30.85	11.50	16	53
Cancer	10	58.80	6.05	48	68
Pneumonia	10	23.90	5.93	17	34
Normal	10	30.40	11.65	16	53
Total	50	34.96	15.53	16	68

P value < 0.05.

The table shows that the age of patients ranged from 16 to 68 years with a mean ±SD 34.96 ± 15.53, Age was ranged from 16 to 53 years with a mean ±SD 30.4 ± 11.56 in group 1, from 16 to 53 years with a mean ± SD 30.85 ± 11.50 in group 2, from 48 to 68 years with a mean ±SD 58.80 ± 06.05 in group 3, and from 16 to 68 years with a mean ±SD 23.90 ± 15.53 in group 4.

Regards to the clinical presentations of the patients; cough is the most common symptom in all (40) patients (100%), expectoration was present in (31) patients 62%, Dyspnoea was present in (18) 36%, Heamoptysis was present in (16) patients 32%, chest pain was present in (15) patients 30% and fever was present in patients (23) 46%.

Table 2. Serum ADA between different groups.

	No.	Mean	SD.	Minimum	Maximum
Tuberculosis	20	47.5	20.05	25.7	97.4
Adenocarcinoma	4	26.15	5.70	21	34.2
Squamous cell carcinoma	6	18.55	3.95	14	25.3
Pneumonia	10	24.87	8.49	12.1	39
Normal	10	23.82	8.24	12.1	37.4

Serum ADA level was (25.7 – 97.4) u/l in tuberculosis patients with mean ± SD 47.5±20.05; (34.2-21) u/l with mean ± SD 26.15±5.70 in patients with Adenocarcinoma; (14.0–25.3) u/l with mean ± SD 18.55± 3.95 in patients with squamous cell carcinoma; (12.1–39) with mean ± SD 24.87 ± 8.49 in patients with pneumonia and (12.1–37.4) with mean ± SD 23.82 ± 8.24 in normal group.

Table 3. BALF ADA between different groups.

	No.	Mean	SD.	Minimum	Maximum
Tuberculosis	20	5.06	1.93	2.70	8.30
Adenocarcinoma	4	1.9	0.53	1.30	2.40
Squamous cell carcinoma	6	2.6	1.47	1.40	5.40
Pneumonia	10	1.77	0.91	0.00	3.10
Normal	10	1.92	0.55	1.10	2.60

BALF ADA level range was (2.7 – 8.3) u/l in tuberculosis patients with mean ± SD 5.06±1.93; (1.30-2.40) u/l with mean ± SD 1.9±0.5 in patients with Adenocarcinoma; (1.4–5.4) u/l with mean ± SD 2.6± 1.4 in patients with squamous cell carcinoma; (0.0 – 3.1) with mean ± SD 1.7 ± 0.9 in patients with pneumonia and (1.1 – 2.6) with mean ± SD 1.92 ± 0.5 in normal group.

The cut-off points resulted when median values used for the entire group i.e. 50 cases was: For serum ADA level, the group median was 26.2 U/L, For bronchoalveolar lavage fluid (BALF) ADA level, the group median was 2.5 U/L, ADA serum sensitivity was 95%, ADA serum specificity was 83.3%, ADA serum Positive predictive value was 79.2%, ADA BALF sensitivity was 100%, ADA BALF specificity was 83.3% and ADA BALF Positive predictive value was 80%.

Table 4. Comparison of serum ADA in pulmonary tuberculosis and the other groups.

		ADA serum (U/L)	
		> 26.2	**≤ 26.2**
Pulmonary TB	Count	1	19
	% within ADA Serum grouping	3.8%	79.2%
Lung cancer	Count	9	1
	% within ADA Serum grouping	36.6%	4.2%
Pneumonia	Count	8	2
	% within ADA Serum grouping	30.8%	8.3%
Normal	Count	8	2
	% within ADA Serum grouping	30.8%	8.3%

Comparison of serum ADA in pulmonary tuberculosis and other groups that was 79.2% in pulmonary tuberculosis, 4.2% in lung cancer, 8.3% in Pneumonia and 8.3% in Normal group.

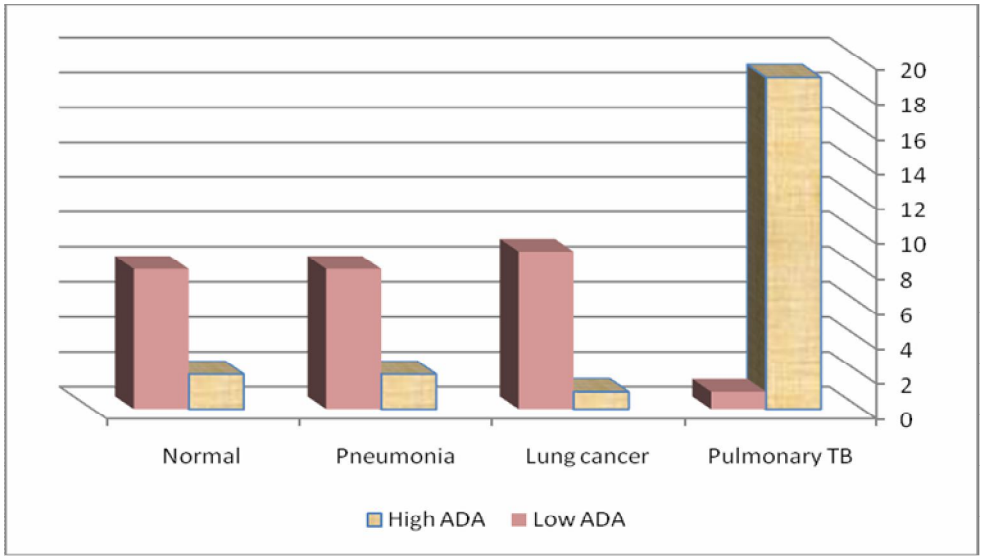

Fig 1. Shows the marked elevation of serum ADA among Tuberculosis patients in comparison to the other groups.

Among 20 patients diagnosed as pulmonary tuberculosis, 19 patients shown elevation of serum ADA level, one patient among 10 patients diagnosed as lung cancer, 2 patients among 10 patients diagnosed as pneumonia shown elevation of serum ADA level and 2 among 10 normal subjects shown elevation of serum ADA level, with sensitivity and specificity 95% and 83.3% respectively.

Serum ADA level was measured among four groups the results were:

- Serum ADA level was (25.7 – 97.4) u/l in tuberculosis patients with mean ± SD 47.5±20.05, (21 - 34.2) u/l with mean ± SD 26.15±5.70 in patients with adenocarcinoma;
(14.0 – 25.3) u/l with mean ± SD 18.55± 3.95 in patients with squemous cell carcinoma, (12.1 – 39) with mean ± SD 24.87 ± 8.49 in patients with pneumonia and (12.1 – 37.4) with mean ± SD 23.82 ± 8.24 in normal group.
- The cut-off points obtained from the median values for all the group i.e. 50 cases, for serum ADA cut off point was 26.2, with sensitivity and specificity 95%, 83.3% respectively and positive predictive value 79.2%.

Table 5. Comparison of BALF ADA in pulmonary tuberculosis and other groups.

		ADA BALF (U/L)	
		> 2.5	**≤ 2.5**
Pulmonary TB	Count	0	20
	% within ADA BALF grouping	0	80%
Lung cancer	Count	8	2
	% within ADA BALF grouping	32%	8%
Pneumonia	Count	8	2
	% within ADA BALF grouping	32%	8%
Normal	Count	9	1
	% within ADA BALF grouping	36%	4%

This table shows Comparison of BALF ADA in pulmonary tuberculosis and other groups that was 80% in pulmonary tuberculosis, 8% in lung cancer 8 % in Pneumonia and 4 % in normal group.

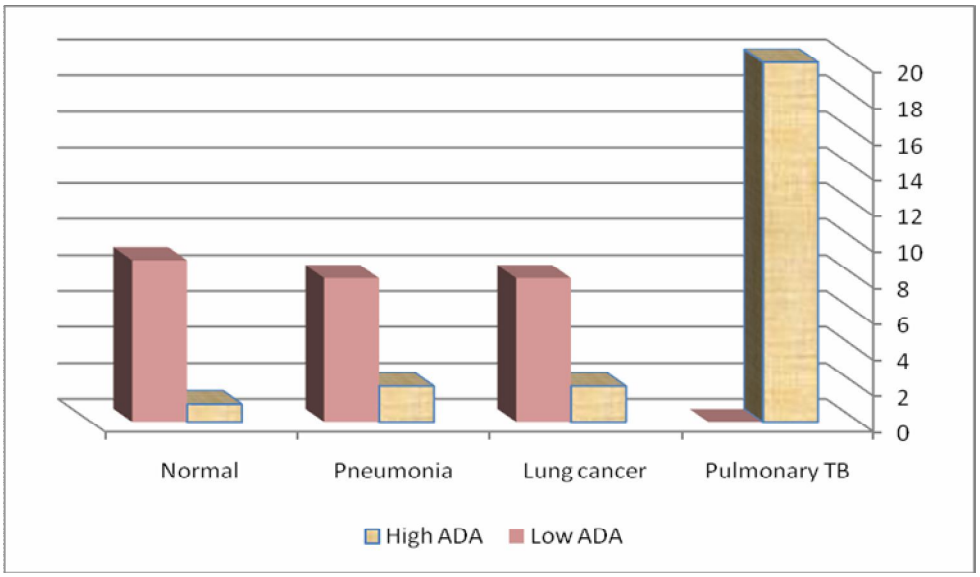

Fig 2. Shows the high elevation of BALF ADA in tuberculosis patients in comparison to the other groups.

Among 20 patients diagnosed as pulmonary tuberculosis, 19 patients shown elevation of BALF ADA level, 2 patients among 10 patients diagnosed as lung cancer show increased ADA level in BALF, 2 patients among 10 patients diagnosed as pneumonia shown elevation of BALF ADA level and one among 10 normal subjects shown elevation of BALF ADA level, with sensitivity and specificity 100% and 83.3% respectively.

BALF ADA level was measured among the four groups the results were:

- BALF ADA level was (2.7 – 8.3) u/l in tuberculosis patients with mean ± SD 5.06±1.93, (1.30- 2.40) u/l with mean ± SD 1.9±0.5 in patients with Adenocarcinoma ;(1.4 – 5.4) u/l with mean ± SD 2.6 ± 1.4 in patients with squamous cell carcinoma, (0.0 – 3.1) with mean ± SD 1.7 ± 0.9 in patients with pneumonia and (1.1 – 2.6) with mean ± SD 1.92 ± 0.5 in normal subjects.
- The cut-off points obtained from the median values for all the group i.e. 50 cases, For BALF ADA, cut off point was 2.5, BALF ADA sensitivity and specificity were 100%, 83.3% respectively with positive predictive value 80%.

Table 6. Lymphocytes.

Maximum	Minimum	SD	Mean	N	diagnosis
65	17	12.64	37.45	20	Pul. TB
50	16	15.17	32.75	4	Adenocarcinoma
65	12	19.40	27.67	6	Squamous cell carcinoma
65	12	17.11	29.70	10	Pneumonia
65	12	17.11	29.70	10	Normal
65	12	15.42	32.80	50	Total

Table 7. Comparison of Lymphocytes between TB and other groups.

		Sig.	Std. error	Mean difference
Pul. TB	0.998	8.51	4.70	Adenocarcinoma
	0.904	7.24	9.78	Sq. cell car.
	0.877	6.02	7.75	Pneumonia
	0.877	6.02	7.75	Normal

P value = 0.526.

The Tables 6,7 show that the lymphocytes in serum of patients ranged from 17% to 65 % with a mean ±SD 37.45 ± 17.11

Lymphocytes was ranged from 17 to 65 % with a mean ±SD 37.45 ± 12.64 in group 1, from 12 to 50 % with a mean ±SD 32.75 ± 15.17 in group 2, from 12 to 65 years with a mean ±SD 29.70 ± 17.11 in group 3, and from 12 to 65 years with a mean ±SD 29.70 ± 17.11 in group 4, No significant difference was observed in mean lymphocytes activity in serum of different groups among all selected patients.

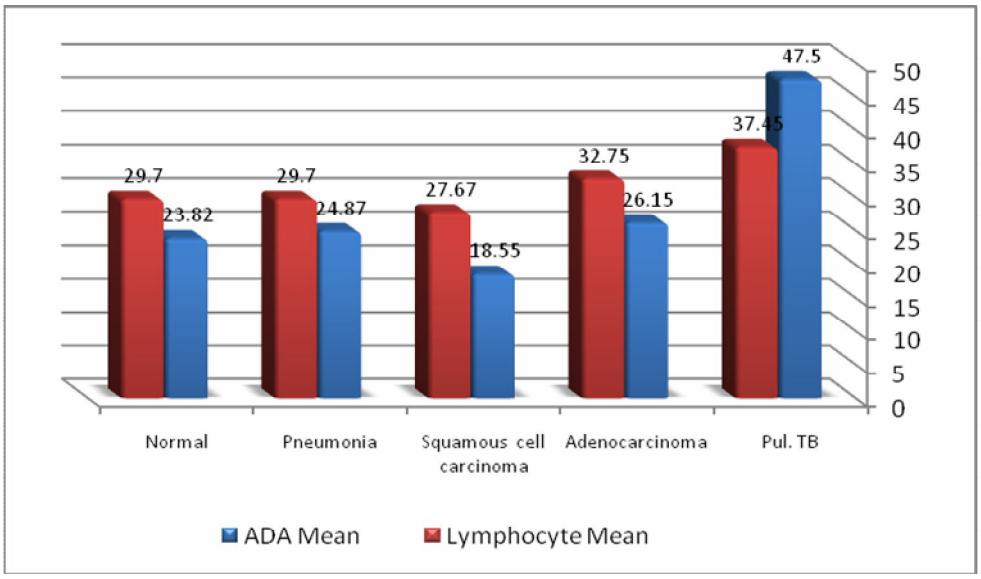

Fig 3. Lymphocytes & ADA mean.

(Fig. 3) shows significant increase at the ADA levels in cases of pulmonary tuberculosis due to increase in the lymphocyte cells.

It seems reasonable to regard the high ADA activity in tuberculous patients as a reflection of local activation of T lymphocytes. The level of ADA activity in tuberculosis is higher than any other disease.

DISCUSSION

We evaluated 50 Egyptian Subjects, 38 (76%) of patients were male and 12 (24%) were female. the age of patients ranged from 16 to 68 years with a mean ±SD 34.96 ± 15.53. (16 to 53 years with a mean ±SD 30.4 ± 11.56 in group 1 and from 16 to 53 years with a mean ±SD 30.85 ± 11.50 in group 2 and from 48 to 68 years with a mean ±SD 58.80 ± 06.05 in group 3 and from 16 to 68 years with a mean ±SD 23.90 ± 15.53 in group 4).

The most prevalent complaint among the 50 subjects was cough as it was found in all (40) patients, then expectoration was found in 31(62%) patients, dyspnea in 18 (36%) patients, Heamoptysis in 16 (32%) patients, chest pain in 15(30%) patients, fever in patients 23 (46%) and toxemic manifestations in 14 (28%) patients.

ADA level was evaluated in the serum and broncho alveolar lavage fluid (BALF) of those patients.

Several studies were carried out, have suggested the use of serum ADA levels for the diagnosis of pulmonary tuberculosis.[7-11] The serum ADA levels decrease to normal levels after one month of the initiation of effective treatment. [12] The decrease in serum ADA levels could be due to the normalization of altered lymphocytes turnover induced by tuberculosis highest usefulness of serum ADA is expected in those patients who have higher serum ADA values at presentation.

The significantly ($P<0.05$) high serum ADA values (74.06±18.5 U/L) were found also in children with pulmonary tuberculosis than the healthy children (40.36±12.0 U/L). [13]

The results of this study showed that patients with pulmonary tuberculosis had significantly higher ADA level in serum and BALF than patients with non-tuberculosis lung diseases (lung cancer , pneumonia) and normal persons ($P< 0.001$) with cut off point obtained was ; in serum ADA level was 26.2, with sensitivity and specificity were 95% and 83.3% respectively and positive predictive value was 79.2% and in BALF ADA level, the cut of point was 2.5, with sensitivity and specificity were 100% and 83.3% respectively and Positive predictive value was 80% . ADA serum levels were statistically significantly increased in T.B patients when compared to patients with non- tuberculosis lung diseases and normal persons and did not differ between normal persons and the other lung diseases groups , this conclude that serum and BALF ADA level is a selective marker of immune stimulation in tuberculosis but not in non- tuberculosis lung diseases

Abnormally high levels of serum ADA level in all the patients with pulmonary tuberculosis indicated the serum ADA could be a good diagnostic tool for tuberculosis. [12]

Serum ADA was found a selective marker of immune stimulation in tuberculosis but not in cancer when compared the serum ADA level of pulmonary tuberculosis patients with the patients of lung cancer pre-treatment and healthy individuals.[14] With cut off values of 30 U/L the specificity, sensitivity, positive predictive values and negative, predictive value of the serum ADA was found to be 90, 87, 90 and 66.5%, respectively.[15]

S. Thora showed the raised activity of adenosine deaminase in the new born sera, six weeks after B.C.G. vaccination.[16] Mishra et al. noticed raised serum adenosine deaminase activity in a group of 51 tuberculous cases compared to 20 healthy individuals.[17] The same results were obtained by Ishii et al. in Japan.[18]

The results of the present study are in agreement with those of Canbolat O. et al, Lakshmiv et al, Orphanidou, Collazos J. et al and Bansal S K. et al, so we can use serum and BALF ADA level to differentiate between highly suspicious pulmonary tuberculosis and pneumonia or lung cancer. That ADA rises in serum and BALF of patients who have pulmonary tuberculosis, so we can use the measurement of ADA in serum and BALF to differentiate pulmonary tuberculosis from other lung diseases.

REFERENCES

1. Mishra OP, Yusaf S, Ali Z, Nath G, Das BK. Adenosine deaminase activity and lysozyme levels in children with tuberculosis. J Trop Pediatr. 2000;46:175-8.

2. TB control report 2009- Global Tuberculosis Control. A SHORT UPDATE TO THE 2009 REPORT. World health organization (WHO) Library Cataloguing-in-Publication Data. "WHO/HTM/TB/2009.426". Accessed at 10 March 2010.

3. Ioanna Samarai, Petros Bakakos, Dora Orphanidou, Pangiota Latis , Sofia Katsimpoula, Georgios S. et al, Adenosine Deaminase Activity in Patients with Pulmonary Tuberculosis and Lung Cancer, Adv Clin Exp Med. 2007;16:533–5.

4. Ungerer JP, Oosthuizen HM, Retief JH, Bissbort SH. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. Chest. 1994;106:33-7.

5. Abdel- Hakim AM, Badrawy H, Gemaei S, Ragab G.M., Helmy N, Soliman MA. Bronchoalveolar lavage cellularity in uncontrolled insulin dependant diabetics. EGYPT J. Chest Dis. Tub. 1989;36:93.

6. Giusti G, Galanti B. Colorimetric method. In: Methods of enzymatic analysis. Eds.: Bergmeier H, Weinheim Verlag Chemie. 1984;3rd:315–23.

7. Collazos J, P. Espana, J. Mayo, E. Martinez and F. Izquierdo, Sequential evaluation of serum adenosine deaminase in patients treated for tuberculosis. Clin. Invest. 1998;114:432-5.

8. Baganha MF, A Pego, MA Lima, EV Gaspar, AR Cordeiro. Serum and pleural adenosine deaminase: correlation with lymphocyte population. Chest. 1990;97:605-10.

9. Bansal SK, RP Singh, RK Narang, LD Joshi, A Bansal, AK Agrawal. Serum adenosine deaminase in pulmonary tuberculosis; malignancy and non-tubercular respiratory tuberculosis. Indian J. Chest. Dis. Allied Sci. 1991;33:189-93.

10. Bhargava DK, M Gupta, S Nijhawan, S Dasarathy, AK Kushwaha. Adenosine deaminase (ADA) in peritoneal tuberculosis: Diagnostic value in ascitic fluid and serum. Tubercle. 1990;71:121-6.

11. Collazos J, Espana P, Mayo J, Martinez E, Izquierdo F. Sequential evaluation of serum adenosine deaminase in patients treated for tuberculosis. Chest. 1998;114:432-5.

12. Conde MB, SR Marinho, F Pereira-Mde, JR Lapae-Silva, MH Saad, CL Sales, et al. The usefulness of serum adenosine deaminase 2 (ADA-2) activities in adults for the diagnosis of pulmonary tuberculosis. Respir. Med. 2002; 96:607-10. J. Med. Sci. 2003;3:30-45.

13. Ida T, S Taniai, M Nitta, J Shimase, K Makiguchi, I Miyasato, et al. Serum adenosine deaminase activity in patients with active pulmonary tuberculosis. Kekkaku. 1990;65:477-81.

14. Kuyucu K, C Karakurt, E Bialaloglu, T Karacan, T Tezic. Adenosine deaminase in childhood pulmonary tuberculosis: diagnostic value in serum. J. Trop. Paediatr. 1999;45:245-7.

15. Lakshmiv, Rao RR, Joshi N, Rao PN. Serum adenosine deaminase activity in bacillary or paucibacillary pulmonary T.B., Indian J pathol Microbiol. 1992;35:48-52.

16. Thora S, Rajsekaran P, Chhaparwal BC. Serum adenosine deaminase estimation in relation to BCG vaccination. Indian Pediatr. 1995;32:1087-8.

17. Ishii S, Nagasawa H, Tai H, Noda Y, Akiyama K, Takeda H, Okajima N, Okano H. Relationship between the activity of serum adenosine deaminase including its isozymes and lymphocyte subpopulation in patients with pulmonary tuberculosis, Kekkaku. 1997;72:153-9. Japanese.

18. Mishra OP, S Yusaf, Z Ali, G Nath, BK Das. Adenosine deaminase activity and lysozyme levels in children with tuberculosis. J. Trop. Paediatr. 2000;46:175-8.