Original Article

Bronchial Asthma and Serum Adenosine Deaminase Activity in Iraqi Patients

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Summary:

Background: *Adenosine deaminase (ADA) catalyzes the irreversible deamination of adenosine in the purine degradation pathway,, and it also inactivates a number of ant-viral and cancer therapeutic analogues It is vital to the proper function of immune system , Adenosine , one of the main substrates for ADA , is known for its bronchoconstrictor and anti arrhythmic actions Some workers have reported an increase in ADA activity in the RBC of asthmatic patients .*

Objective: *The aim of this study was to measure the activity of AdA in serum of asthmatic Iraqi Patients of different ages & duration of their asthma. Mareover , the effects on this enzyme of the different drug treatment of bronchial asthma were studied.*

Subjects & methods: *Adenosine deaininase activity was measure in sera of 71 patients with bronchial asthma and compared to 62 controls . Serum ADA activity was assayed by the sensitive coloimetric methods of Galanti & Giusti.*

Results: *Serum ADA activity was significantly increased in asthmatic patients ($p < 0.01$) when compared to controls. ADA activity was independent of age or sex in both normal and asthmatics. It was also independent of the type of drug treatment. Purification of serum ADA in asthmatics by gel filtration- chromatography revealed increased activity of isoenzymes 4 and 5 .*

Conclusion: *Measurement of serum ADA and its isoenzymes may be used markers in diagnosis of asthma and is uninfluenced by the available drug treatment of asthma or its duration.*

Key Words: *Adenosine deaminase activity, isoenzymes, Bronchial asthma.*

Introduction

Adenosine deaminase (ADA) catalyzes the irreversible deamination of adenosine in the purine degredation pathway, and it also inactivates a number of anti - viral and cancer therapeutic analogues (1, 2.3). It is vital to the proper function of immune system , being essential for growth and proliferation of T-lymphocytes as well as maturation of monocytes to macrophages ; it also protects lymphocytes from the toxic effect of high concentration of ADA substrate^ 4 ,5, 6). Adenosine , one of the main substrates for ADA , is known for its bronchoconstriccting and anti-arrhythmic actions (7). Some workers have reported an increase in ADA activity in RBC of asthmatic patients (8). In order to elaborate on this, the present work was designed to measure ADA activity in serum of asthmatic Iraqi patients of different ages and duration of their asthma. Moreover, the effects of different drug treatment for bronchial asthma were studied

PATIENTS AND METHODS :

A total of 133 subjects were included. They were 71 asthmatics , 38 males and 33 females , ranging in age from 4 to 75 years (mean age 36 y), and 62 controls (34 males, 28 females) ranging in age from 7 to 55 years (mean age of 38 y).

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Asthmatic patients were of 3 groups

a : Untreated (5 patients)

b : Treated acutely with aminophylline (8 patients)

c : Treated chronically with single or multiple drugs for asthma

as follows:

1 - salbutamol (16 patients)

2 - aminophylline + prednisolone or hydrocortisone (14patients)

3 - salbutamol + aminophylline (5 patients)

4 - salbutamol + prednisolone (8 patients)

5 - salbutamol + theoplylline + prednisolone (15 patients)

The duration of asthma varied from 6 month to 27 years (41 patients less than 10 years duration , 21 patients between 10 to 20 years duration , and 9 patients more than (9 years duration)

ADA Assay in Serum

5 ml blood samples were withdrawn from each subject and were allowed to coagulate at room temperature before being centrifuged at 3000rpm for 10 minutes. & the resulting sera were separated.

Blood samples were collected, whenever possible, before treatment of acute attack of asthma with aminophyiline, and after treatment of acute attacks.

Serum ADA activity was assayed by the sensitive colorimetric method of Galanti and Giusti (9), with adenosine being used as a

substrate. The ammonia produced forms an intensely blue indophenol with sodium hypochlorite and phenol alkaline solution . Ammonia concentration is directly proportional to the absorbance of the indophenol at 630 nm .

One international unit (I.U) of ADA activity is defined as the amount of enzyme that catalyzes the deamination of one micromole adenosine per minute at 37C under standard assay conditions in 1 litre serum .

Purification of ADA in asthmatics' serum

Separation and purification of serum ADA were done by gel filtration-chromatography (11), employing column (67x1.6cm) of sephacryl S 200 resin , using phosphate buffer 0.05 mM, pH 6.5 . The crude enzyme was diluted by a minimum volume (1.5ml) to give a protein concentration of 32 mg/ml(1 2) .

Statistics :-

Comparisons were made by the students t - test for independent data unless otherwise indicated.

Results:

ADA activity in Asthma

The mean ADA in serum of asthmatic patients was 27. 47 U/L, which was significantly higher than that of normal controls (18.034 U/L) ($P < 0.001$) (table 1). There was no significant difference in ADA activity between males and females in both asthmatics and normal controls (Fig .1). Similarly, no significant difference was seen in enzyme activity between different age groups in normal and in asthmatics. (Fig .2). Regarding disease duration, no significant change was seen except where duration exceeded 20 years in males . (Fig.3).

Effect of drug treatment

Acute drug treatment with aminophyliine IV induced a non-significant reduction in serum ADA activity (fig.4)($p > 0.05$). Similarily, no significant change in ADA activity occurred with different drug groups on chronic treatment for their asthma

ADA Purification :

This yielded five isoenzymes; the peak for the third isoenzyme is less than that shown for normal; the fourth isoenzyme was twice the activity of normal serum, while the fifth isoenzyme in asthmatics had the highest activity of about 5 time that of normal serum (table 2).

DISCUSSION

It could be seen that ADA activity in plasma is higher in patients with bronchial asthma than in normal controls, and that this increase is independent of age , sex or disease duration . Other workers reported increased ADA activity in RBC of asthmatics but our work is the first to show this increase in the serum of asthmatics; this higher ADA activity may be related to the higher adenosine level reported in asthmatics (13). It might be that adenosine inhibits activity of suppressor T - lymphocytes which would perpetuate the immune reaction in asthma, and in that sense, the increase in ADA activity is protective by reducing adenosine accumalation in asthmatics. Although excess adenosine causes bronchoconstriction, its pkysiological level inhibits IgE-induced release of histamine from basophils or mast calls, probably by being utilized to increase cAMP level in these cells (14). It should be noted that conversion of ATP into cAMP would result in reduced adenosine formation which might result in a secondary decrease in ADA activity. The different drugs used by bronckal asthma patients had no significant effect on ADA activity in patients' sera. Salbutamol increases cAMP level by stimulation of B2 -adrenergic receptors in bronchial smooth muscle (15), while theophyllines including aminophylline inhibits phosphodiestrase enzyme that catalyzes cAMP catabolism. In addition , theoptyllines act as competitive antagonists of adenosine at adenosine receptors (16) , and inhibits free superoxide radicals release from eosinophils (17) Hydrocortisone stabilizes the membranes of different reactive cells , and may enhance the action of bronchoditators like sulbutamol or theoplylline in asthmatic However high concentration of these drugs in vitro directly inhibited ADA enzyme activity. Furthermore, it was shown that this inhibition is competitive in case of salbutamol and hydrocortisone and of mixed type with aminophylline (unpublished data) (18).

The purification of ADA in asthmatics' serum revealed 5 isoenzyme peaks. This is done for the first time in asthma, and comparing our isoenzymes with their normal profile in normal serum, as done by Al -Nify (19), showed that isoenzymes 4 and especially 5 are much higher than in normal subjects and they might be of diagnostic value or as markers in asthma. This increase may be secondary to the increase in adenosine in asthmatics and possibly also due to leak of these isoenzyme from inflammatory cells accumulating in bronchi in asthmatics.

In conclusion , it seems that the increased ADA in asthmatic patients is independent of age , sex , duration of disease, or type of drug treatment.

Purification of ADA revealed increased activity of isozymes 4 and 5, which could serve as a useful diagnostic marker in early asthma and to differentiated it from disorders of psychogenic, respiratory, or cardiac origin.

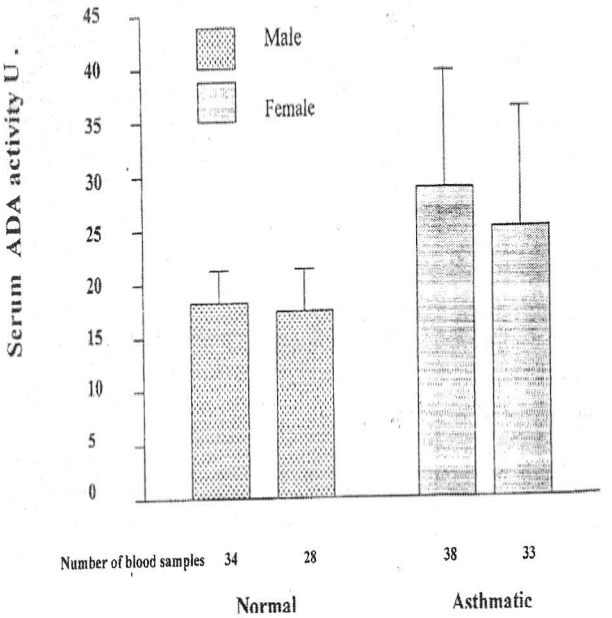


Figure (1) : Serum ADA activity of asthmatic patients and normal healthy controls in accordance with sex (mean ± SD).

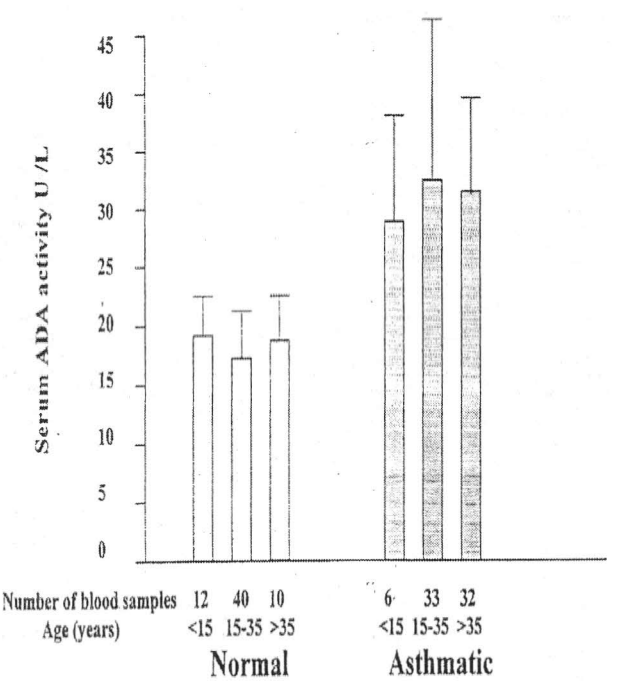


Figure (2) : Serum ADA activity of asthmatic patients and normal healthy controls in accordance with age (mean ± S.D) .

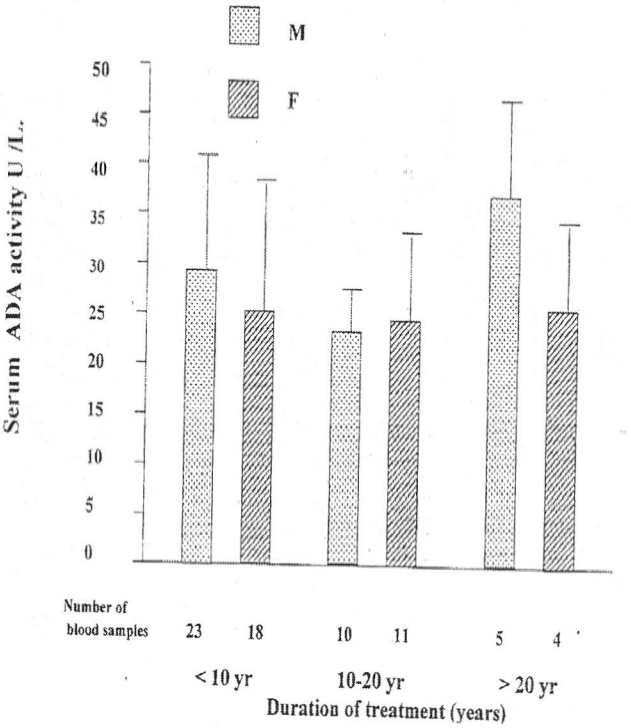


Figure (3) : Serum ADA activity in accordance with duration of chronic treatment and sex (mean ± S.D) .

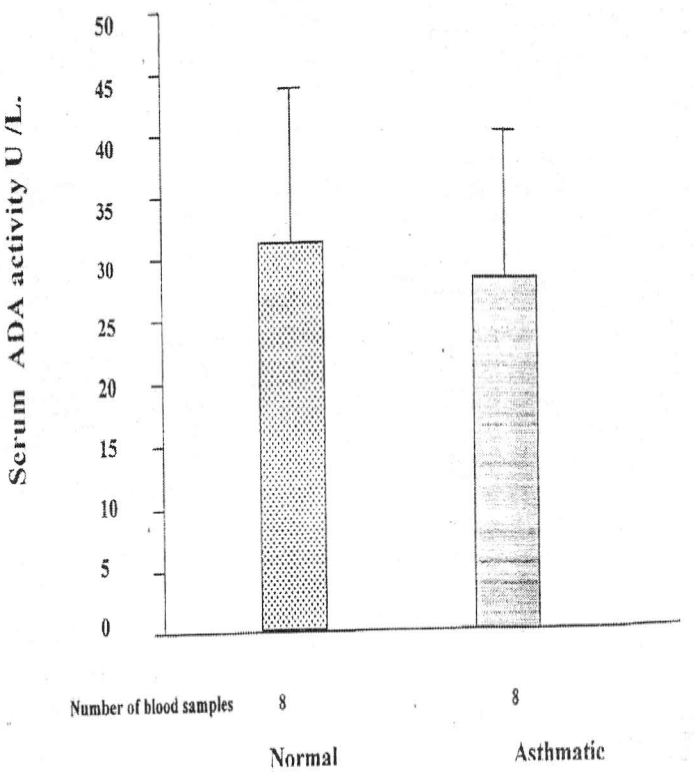
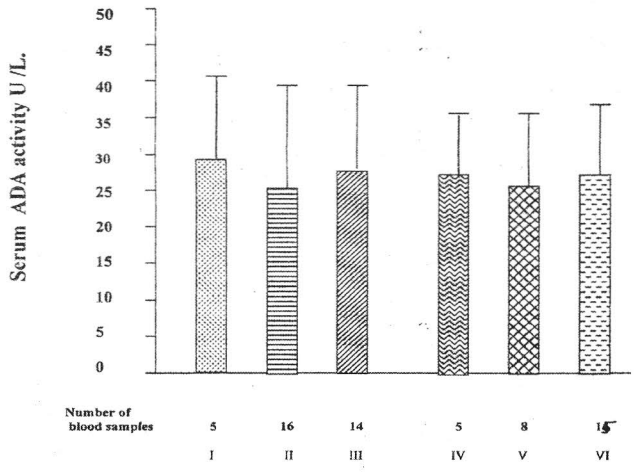


Figure (4): Serum ADA of asthmatic patints before and after acute treatment with aminophylline i.v (mean +S.D)



I Untreated asthamatics
II Salbutamol
III Aminophylline + (hydrocortisone/prednisolone)
IV Salbutamol + theophylline
V Salbutamol + prednisolone
VI Salbutamol + (theophylline + prednisolone)

Figure (5) : Serum ADA activity of asthmatic patients using different combinations of chronic treatment (mean ± S.D) .

	Volume of fraction (ml)	Concentration of protein (mg/ml)	Total amount of protein (mg)	Activity (units/ml)	Specific activity (units/ mg protein)	Total activity (unit)
Crude enzyme	1.5	32.000	48.000	0.016	5×10^{-5}	0.024
Isoenzyme 1	2	0.074	0.148	9.770×10^{-5}	0.013	19.540×10^{-5}
Isoenzyme 2	2	0.100	0.200	1.465×10^{-4}	0.015	2.929×10^{-4}
Isoenzyme 3	2	0.172	0.344	2.920×10^{-4}	0.017	5.858×10^{-4}
Isoenzyme 4	2	0.148	0.296	2.441×10^{-4}	0.016	4.882×10^{-4}
Isoenzyme 5	2	0.035	0.070	5.859×10^{-4}	0.167	11.718×10^{-4}

Table (2): Purification of asthmatic serum ADA by gel filtration chromatography using Sephacryl S-200

	Controls (A)	Asthmatics (B)
Sample size (n)	n l = 6 2	n2 = 71
Mean	xl = 18.034	x2 = 27.470
Standard deviation	si =3.734	s2= 11.250
Standard error	s x l = 0.474	sx2 = 1.335
Range	11.300-25.625	12.695-74.707

t - test between A/B = P < 0.001

Table (1): Serum ADA activity (I.U/L) in asthmatic patients and control group.

References:

1 - Agarwal R.P., Cha S., Crabtree G.W., and Parks R.E. Coformycin tight - binding inhibitor of adenosine deaminase. In : Harmon R.E. et al. Chemistry and Biology of Nucleotides. Academic press Inc., 157-159.

2 - Warburg O. and Christian, W. Biochem Z ., 1941 , 310 , pp.384.

3 - Harriman G.C.B. and Abushanab, E. Adenosine deaminase inhibitors . J.Med. Chem., 1994, 37(2), 307 - 308 .

4 - Petterson T., Ojala K. and Weber T.H. Adenosine deaminase in diagnosis of pleural effusions . Acta Med. Scand, 1984, 215,299-304.

5 - Mejer J., Horbou S. andNycardP. Purine metabolizing enzymes in lymphocytes from patients with solid tumors. Acta Med .scand. 1984,215,5-11

6 - Taher U.Z. , Habibulah CM. and Saleem Y. Decreased adenosine deaminase activity in peripheral lymphocytes of patients sufferring from amoebic liver abscess Arch. Med. Res., 1993 ,24 (2), 203-204.

7 - Laurence D.R. , Bennett P.N. and Brown M.J. in :Clinical Pharmacology 8th edition . 1997, Ch .25, PP.476 Churchill Lisingstone.

8 - Hwang K.C. , Wang J.Y. and Hsieh K.H. Increased erthrocyte adenosine deaminase activity in asthmatic children . Acta Pae'diatr. Sin. 1990 , 31 (2) ,76-80 .

9 - Giusti, G. Adenosine deaminase. In : Methods of Enzymatic analysis. Second ed., vol. 2, Ed. Bergmeyer, H., Academic press, 1092 -1099.

10 - Goodman L.S., Gilman A.G., Rail T.W. and Murad F. in pharmacological Basis of therapeutics. 7th edition , 1985 , Macmillan Publishing Company.

11 - Agarwal E.P. and Parks R.E. Adenosine deaminase in human erythrocyte . Methods in Enzymology, Academic press N.Y 1978, 503-507.

12 - Lowry O.H. et al. Protein estimation . J.Biol.Chem., 1951, 193,265

13 - Bradys T.G. and Odonovan C.I. A study on the tissue distribution of adenosine deaminase in sexes of mammalian species. Comp. Biochen. physiol 1965 , 14 , 101 - 120 .

14 - Marone G., Findlay R.S., and Lichtenstien L.M. Adenosine receptors on human basophils : modulation of histamine release. J. of Immunol., 1979 , 123(4), 1473 - 1477 .

15 - Weiner, N. Norepinphriile,. epinephrine, and sympathomimetic amines. In : Pharmacological basis of Therapeutics.7 th ed., eds. Goodman Gilman, Rail and Murad, 1985 , 145 - 180 . Macmillan Publishing company, New York .

16 - Rail . T.W. Central nervous system stimulants : The Methylxanthines. In : Pharmacological basis of Therapeutics.7th ed., eds. Goodman , Gilman , Rail and Murad, 1985 , 145 -180 . Macmillan Publishing company, New York .

17 - Yukawa T. et al. Effect of. theophylline and adenosine on eosinophil function. Am. Rev. Resp. Dis., 1989, 140, 327 - 333

18 - Abd Alia B.M. A prospective study on adenosine deaminase activity in sera of 71 patients with bronchial Asthma in Baghdad. M.Sc. thesis, submitted to University of Baghdad, 1999, PP. 54-57

19 - Al -NifyS.M. Studies on adenosine deaminase enzyme in sera of typhoid fever and other related liver disease patients. M.Sc. thesis., 1980, submitted to College of Science, Univ. of Baghdad.