STUDIES ON THE AGE DEPENDENT CHANGES IN SERUM ADENOSINE DEAMINASE ACTIVITY AND ITS CHANGES IN HEPATITIS

K. C. Vasudha, A. Nirmal Kumar, T. Venkatesh*

Department of Biochemistry, Bangalore Medical College, Bangalore - 560 002.

* Department of Biochemistry & Biophysics, St. John's Medical College, Bangalore - 560 034.

ABSTRACT

Serum Adenosine deaminase (ADA) activity in normal healthy control subjects increases upto 30 years, remains steady between 31-60 years of age and shows a steep increase in the age group of 61-70 years. This was compared with serum aspartate transaminase (AST) and alanine transaminase (ALT) activity which also showed a gradual increase upto 40 years of age and decreased thereafter. The activities of serum ADA, AST and ALT increased in patients with hepatitis of all age groups compared to their respective controls. The degree of increase in the activities of the above enzymes in hepatitis, decreased with age. The present study also shows that while studying serum ADA activity in hepatitis for diagnostic purposes, the value obtained in a particular age group should be compared with normal range of values for the respective age group only.

KEY WORDS

Serum, ADA, Hepatitis, Aging

INTRODUCTION

The diagnosis of organ disease is aided by measurement of a number of non-functional plasma enzymes characteristic of that tissue or organ. The amount of enzymes released depends on the degree of cellular damage, the intracellular concentrations of the enzymes and the mass of affected tissue. The cause of the damage (viral infection, hypoxia, surgical, chemical, or mechanical trauma) has no bearing on the enzymes released into circulation. The concentration of the enzymes released reflects the severity of the damage. Mild inflammatory conditions are likely to release cytoplasmic enzymes whereas necrotic conditions yield mitochondrial enzymes as well. The use of appropriate normal ranges is important in evaluating abnormal levels of plasma enzymes. The normal range is affected by a variety of factors like age, sex, race, degree of obesity, pregnancy, alcohol, drug consumptions and malnutrition.

ADA (EC 3.5.4.4) activity was first noticed by Gyorgy and Rothler. The enzyme is widely distributed and is found in the intestinal mucosa, spleen, liver, skeletal

muscle, kidney, serum, lymphocytes, leucocytes and erythrocytes. ADA catalyses the irreversible hydrolytic deamination of adenosine to produce inosine and ammonia. Inosine is then further degraded to uric acid or salvaged at several points for the biosynthesis of purines. Ammonia is detoxified either by conversion to urea or glutamine.

The normal values for serum ADA in humans have been studied by various workers and found to range from 2.38 ± 1.43 U/L to 17.0 U/L. Elevated serum ADA activity with increased serum levels of AST, ALT and immunoglobulins were reported in portal cirrhosis (1, 2), hepatitis (3, 4), jaundice and liver fibrosis (5), hepatoma (6) and acute infective hepatitis (7).

Aging is a multi-factorial phenomenon involving various levels of organization leading to a gradual decline in the functional capacity of an organism. Enzymes catalyse specific biochemical reactions in the body. Changes in their levels and properties alter the functional ability of an organism. Hence, enzyme levels are observed depending on sex and diet. Enzymes also exhibit *circadian rhythms*. In spite of all these limitations, it has been shown that some enzymes decrease with age, some increase and others remain a constant (8, 9). Very few studies on age related changes in serum non functional enzymes have been reported.

The objectives of this study were to determine the serum ADA activity in normal individuals of different age groups and also in hepatitis in different age groups

Author for Correspondence :

Dr. K.C. Vasudha,
Associate Professor,
Department of Biochemistry,
M.S. Ramaiah Medical College,
Bangalore - 560 054
Email : vasudhachokkanna@yahoo.co.in

for comparison with normal ADA activity in those age groups and also with other known markers of hepatitis like serum bilirubin, serum ALT and AST; serum total proteins and A/G ratio, to see if this estimation could serve as an useful and sensitive test in the early diagnosis of hepatitis.

MATERIALS AND METHODS

a) The chemicals used were from the following sources: Adenosine (Loba Chemies Pvt Ltd); BSA (SIGMA); Sodium pyruvate (Koch-light Laboratories Ltd); Alanine, Aspartate and 2:4 DNPH (BDH). The other chemicals were of analytical grade made locally.

The study was carried out at Bangalore Medical College and Victoria Hospital. Patients who came for routine investigations to the hospital and who were found to be normal, belonging to different age groups were chosen for the control serum samples.

Serum samples were also collected from patients who were clinically diagnosed as cases of hepatitis with total bilirubin levels above 1.2 mg/dl. Control group as well as hepatitis group comprised of 105 patients each with 15 patients in each age group. Serum AST, ALT, total protein and A/G ratio were estimated for comparison with serum ADA levels.

Venous blood was collected. Serum samples free from haemolysis was used.

The samples were stored below 4°C and the estimates were carried out within 72 hours.

- Serum bilirubin - method of Malloy and Evelyn, 1937 using Vandenberg's reaction (10).
- Serum adenosine deaminase - Colorimetric method described by Guiseppe Guisti 1974 (11).
- Serum aspartate aminotransferase and serum alanine aminotransferase - Method of Reitman and Frankel, 1957 (12)

- Serum total proteins and A/G ratio - Biuret method [Reinhodl, J.G: 1953] (13)

Statistical Analysis : Statistical analysis of the results were done using the students 't' test (14) and statistical software namely SPSS 10.0 and systat 8.0

RESULT AND DISCUSSION

Table 1 shows the comparison of ADA in U/L in the control and hepatitis group. It is observed that normally from 0-30 years, there is a gradual increase in the activity of serum ADA; from 31-60 years, the activity is almost steady and there is a steep rise in the activity above 60 years of age.

Therefore the mean value of serum ADA activity in normal healthy persons is found to be 5.12 U/L - 20.07 U/L. The data closely agrees with earlier results (15, 16).

The serum ADA activity is significantly enhanced ($p < 0.01$) in patients with hepatitis compared to normal healthy individuals in all age groups. The data agrees with earlier results respectively (3, 5).

Tables 2 and 3 show the comparison of age dependent changes in serum AST and ALT in U/L in control and hepatitis groups. It is observed that both serum AST and ALT activities decrease beyond 41-50 years of age in the control group. Results on serum AST correlates with the observations of Wilson and Tshuchiya (9, 17). Reports on age related serum ALT levels are scanty. Also serum AST and ALT activities are significantly enhanced and ($p < 0.01$) in patients with hepatitis in different age groups as compared to corresponding age groups in the control samples.

The degree of increase in serum levels of the above enzymes in hepatitis is more in younger age groups. Serum ADA activity is increased by 7 times in 0-10 years age group as against 1.5 times in the age group of 61-70 years. Serum AST and ALT activities are

Table 1. Age dependent changes of serum ADA in control and hepatitis groups

Age in years	ADA in U/L		Significance By student t	Magnitude of study effect ω^2
	Normal (Mean $\pm$ SD)	Hepatitis (Mean $\pm$ SD)		
0-10	5.12 $\pm$ 1.88	35.26 $\pm$ 2.47	37.61, $P < 0.01$	0.98
11-20	6.25 $\pm$ 0.94	36.01 $\pm$ 4.02	24.45, $P < 0.01$	0.95
21-30	9.09 $\pm$ 3.10	36.45 $\pm$ 3.70	21.95, $P < 0.01$	0.94
31-40	13.10 $\pm$ 1.81	31.52 $\pm$ 3.10	19.87, $P < 0.01$	0.93
41-50	12.90 $\pm$ 1.18	24.94 $\pm$ 2.47	17.03, $P < 0.01$	0.91
51-60	13.65 $\pm$ 2.27	32.01 $\pm$ 5.40	12.14, $P < 0.01$	0.83
61-70	20.07 $\pm$ 1.92	34.80 $\pm$ 2.83	16.69, $P < 0.01$	0.90

Each value is mean $\pm$ SD for 15 samples.

Table 2. Age dependent changes of serum AST in control and hepatitis groups

AST in U/L				
Age in years	Normal (Mean± SD)	Hepatitis (Mean± SD)	Significance By student t	Magnitude of study effect ω^2
0-10	2.41±0.39	63.8±6.27	37.85, P<0.01	0.98
11-20	7.42±1.66	49.87±6.70	23.82, P<0.01	0.95
21-30	9.98±1.20	56.5±9.97	17.94, P<0.01	0.91
31-40	10.14±1.18	46.10±4.97	27.26, P<0.01	0.96
41-50	5.14±0.90	30.7±7.84	12.54, P<0.01	0.84
51-60	5.47±1.20	25.53±5.25	13.13, P<0.01	0.85
61-70	6.21±0.45	37.25±7.91	15.17, P, 0.01	0.88

Each value is mean ± SD for 15 samples.

Table 3. Age dependent changes of serum ALT in control and hepatitis groups

ALT in U/L				
Age in years	Normal (Mean± SD)	Hepatitis (Mean± SD)	Significance By student t	Magnitude of study effect ω^2
0-10	2.89±0.78	44.16±5.62	28.17, P<0.01	0.96
11-20	8.93±1.68	45.47±5.74	23.66, P<0.01	0.95
21-30	13.03±0.39	47.45±4.98	26.69, P<0.01	0.96
31-40	13.52±2.36	44.52±4.64	23.06, P<0.01	0.95
41-50	7.40±1.20	27.58±12.3	6.32, P<0.01	0.57
51-60	5.88±0.03	31.53±10.83	9.17, P<0.01	0.73
61-70	6.37±0.43	25.87±7.22	10.44, P<0.01	0.78

Each value is mean ± SD for 15 samples.

increased by 26 times and 16 times respectively in the younger age group but it is only 6 times and 4 times respectively in the oldest age group. Studies of altered adaptive increase in the activities of a number of enzymes in response to broad spectrum of stimuli, in tissues have been used to explain the phenomenon of aging. Generally, it has been observed that the degree of increase in the activity of tissue enzymes in response to any treatment decreases with age chronologically. (6, 7) Magnitude of study effect ω^2 shown in the above tables and Fig. 1 explain the extent to which changes in enzyme levels can demarcate the normal from hepatitis group. Accordingly it is observed that ω^2 is consistent for both serum ADA and AST levels throughout but for serum ALT, ω^2 decreases beyond 40 years of age probably suggesting that serum ADA and AST are better diagnostic markers in the older age group as compared to serum ALT.

Serum total proteins and A/G ratio was also determined in these cases of hepatitis. Serum total proteins showed a mean of 6.63-8.16 g/dl falling well

within the normal range of 6-8 g/dl. On the contrary, serum A/G ratio was found to be significantly reversed showing mean of 0.56:1 to 0.84:1 (normal range being 1.5:1 to 2.5:1). Serum bilirubin levels were also found to be elevated in these cases ranging from 1.6 mg% to 5.7 mg% (normal range being 0.2 mg% - 1.2 mg%). Increase in serum bilirubin levels were compared with increase in serum ADA, AST and ALT activities (Table 4). It is observed that serum AST and ALT activities increase proportionately (R^2 in % = 97 and 93 respectively) with increase in serum bilirubin levels whereas serum ADA shows a steep increase at higher levels of bilirubin (R^2 in % = 89).

SUMMARY AND CONCLUSION

The serum levels of plasma non-functional enzymes normally varies in different age groups for different enzymes. In diagnostic enzymology, the values of the enzymes must be interpreted taking into consideration the age of the patient. In older age groups, serum ADA appears to be a better biochemical marker for hepatitis as compared to serum ALT.

Fig. 1. Comparison of the magnitude of study effect between the three enzymes in hepatitis

Table 4. Comparison of the activity of the three enzymes with Serum bilirubin in hepatitis

Serum Bilirubin levels in mg%	Serum ADA in U/L (Mean±SD)	Serum AST in U/L (Mean±SD)	Serum ALT in U/L (Mean±SD)
1-2	25.32±4.35	19.54±4.56	17.69±1.19
2-3	27.77±5.29	38.72±5.41	37.11±10.34
3-4	29.18±4.94	47.50±5.00	38.65±7.18
4-5	39.56±6.06	57.70±13.91	46.57±3.38
5-6	51.52±4.70	79.83±7.36	60.44±7.02
Type of Increase	(Quadratic) Steep increase at higher level of bilirubin	(Linear) Constant increase at every level of bilirubin	(Linear) Constant increase at every level of bilirubin
R ² in %	89.00	97.00	93.00

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