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ADENOSINE DEAMINASE ACTIVITY IN SERUM OF PATIENTS WITH VIRAL HEPATITIS IN TWO TERTIARY INSTITUTIONS IN SOUTH EASTERN NIGERIA

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ABSTRACT

Serum adenosine deaminase (ADA) activity was assayed along with other liver function indices in viral hepatitis patients at ESUT Teaching Hospital Enugu and Iyienu Mission Hospital Ogidi, between April and October 2015. Sixty (60) patients, 32 males and 28 females were divided into three groups with regards to their serotypes of hepatitis virus infections viz: HBV group (n=37), HCV group (n=17), and HBV/HCV co-infection group (n=6). Sixty (60) healthy subjects, 34 males and 26 females, served as the control group. Clinical diagnosis of patients was confirmed by rapid immuno-chromatographic tests. All subjects were HIV seronegative. Serum ADA activity, other liver enzymes, bilirubin and proteins were assayed by colorimetric methods. Results showed that serum ADA activity in patients with HBV (22.47 ± 11.17U/L) and those with HCV (22.14 ± 8.55U/L) were significantly higher ($p<0.05$) than the values of serum ADA in the control group (10.99 ± 4.69U/L). The Serum activity of AST (18.68 ± 11.87U/L), ALT (21.03 ± 12.38U/L), ALP (85.22 ± 39.13U/L) and T_{BIL} (3.34 ± 4.78µmol/l) in patients with HBV and the mean value of AST (20.84 ± 13.36U/L), ALT (22.29 ± 11.92U/L), ALP (86.76 ± 67.03U/L), T_{BIL} (2.09 ± 1.34µmol/l) in those with HCV were significantly higher ($p<0.05$) than the corresponding values in the control group; AST (9.35 ± 5.27U/L), ALT (5.50 ± 3.25U/L), ALP (43.65 ± 20.92U/L), T_{BIL} (1.48 ± 1.05µmol/l). The C_{BIL} level in patients with HBV (1.20 ± 3.62µmol/l) and those with HCV (0.53 ± 0.47µmol/l) were not significant ($p>0.05$) when compared to the control group (0.27 ± 0.40µmol/l). There was no significant difference in ADA activity between patients with HBV, HCV, and HBV/HCV co-infection ($p>0.05$). A positive correlation was observed between serum ADA activity and those of AST, ALT, TBIL, and CBIL ($p<0.05$) in viral hepatitis infection. We concluded from this study that increase in serum ADA activity corresponding to those of ALT, AST, and Bilirubin is an approximate index for the diagnosis of hepatitis infection, which is the same in patients infected with HBV, HCV or both, and may be a valuable index in diagnosis and monitoring of patients with viral hepatitis.

Keywords: Liver, Hepatitis, detoxification, enzyme, Adenosine

INTRODUCTION

The liver is perhaps the most biochemically complex organ within the body. It possesses enzymes and cofactors necessary for an unparalleled number of metabolic reactions. It is of vital importance in intermediary metabolism and in the detoxification and elimination of toxic substances (Pratibha *et al.*, 2004).

The diagnosis of organ disease is aided by measurement of non functional plasma enzymes characteristic of that tissue or organ. The amount of enzymes released depends on the degree of cellular damage, 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 enzyme released into circulation. The concentration of enzymes released reflects the severity of damage. Mild of inflammatory conditions are likely to release cytoplasmic enzymes where as necrotic conditions yield mitochondrial enzymes as well. The use of appropriate normal range is important in evaluating normal levels of plasma enzymes.

Adenosine deaminase (ADA, adenosine aminohydrolase, E.C.3.5.4.4) is an enzyme involves in the catabolism of purine bases, that catalysis the irreversible hydrolytic deamination of adenosine to produce inosine and ammonia (Fox and Kelly, 1978). 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 enzyme is widely distributed and is found in the intestinal mucosa, spleen, liver, skeletal muscle, kidney, lymphocytes, leucocytes and erythrocytes. ADA is higher in lymphoid tissues and principal biological activity of ADA is detected in T lymphocytes (Sullivan *et al*, 1977). Its main physiological activity is related to proliferation, differentiation and maturation of lymphoid cells (Ma *et al*., 1982). This relationship may have additional significance in T cells, since ADA is directly associated with the extracellular domain of CD 26, which is defined as one of the many T- cell activation antigens (Kameoka *et al*., 1993). As a maker of cellular immunity, ADA serum activity is found to be elevated in diseases in which there is cell - mediated immune response (Galanti *et al*.,

1981; Piras *et al*., 1982). High serum ADA activities are observed in patient with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis and hepatoma (Kobayashi *et al.*, 1993).

RELATED WORKS

ADENOSINE DEAMINASE

Adenosine deaminase (ADA, adenosine aminohydrodase, E.C.3.5.4.4) is an enzyme involved in the catabolism of purine bases, that catalysis the deamination of adenosine, to form inosine and ammonia (Fox and Kelly, 1978). ADA activity is widely distributed in human tissue and is higher in lymphoid tissues, and principal biological activity of ADA is detected in T lymphocytes (Sullivan *et al*., 1977). Its main physiologic activity is related to lymphocytic proliferation and differentiation. As a marker of cellular immunity, its serum activity is found to be elevated in diseases in which there is a cell-mediated immune response (Galanti *et al*., 1981; Piras *et al*., 1982). The enzyme is widely distributed and is found in the intestinal mucosa, spleen, liver, skeletal muscle, kidney, serum, lymphocytes, leucocytes and erythrocytes. ADA activity was first noticed by Gyorgy and Rothler (Vasudha *et al*., 2006).

ADA activity in humans is found to be associated with several different isoenzymes; ADA 1 (40 kDa) is a monomer (designated as one catalytic unit) which can combine with a nonenzymatic 200-kDa, dimeric glycoprotein, designated as a combining protein (cp) Ratech *et al.,* 1981). These ADA isoenzymes are denoted ADA 1 (40 kDa) and ADA 1+cp (280 kDa). Although, these isoenzymes account for greater than 90% of the activity ADA in human cells and tissue, an additional 110-kDa ADA isoenzyme, designated ADA 2, has been reported in the spleen. ADA 2 is a minor component of ADA activity in the spleen and the liver but a major component of the ADA activity in serum (van der Weyden *et*

al., 1976). However, there are basically two isoenzyme of ADA: ADA1 and ADA 2. The isoenzymes ADA 2 coexist with ADA 1 only in monocytes-macrophages. ADA 1 and ADA 2 are coded by different gene loci (Hirschhorn and Ratech, 1980; Zuckerman *et al*., 1980; Gakis *et al*., 1982, 1983). The Km of ADA 1 is 5.2 x 10^{-5}M with optimal pH of 7.0 – 7.5. The Km of ADA 2 is 200 x 10^{-5}M with an optimal of 6.5 (Giusti and Gakis 1971; Hirschhorn and Retech, 1980; Gakis *et al*., 1982). However, since ADA activity in body fluid is due a mixture of ADA 1 and ADA 2, to estimate the precise quantity of each of these isoenzymes in a specimen, one should separate the two isoenzymes. This is highly complicated. Tsuboi *et al.* (1995), reports the specific inhibition of the ADA 1 isoenzymes at 100 µM erythro-9-(2-hydroxy-3- nonyl) adenine (EHNA) in the presence of adenosine substrate, while ADA is not inhibited. ADA 1 activity can therefore be calculated by subtracting the residual activity in the presence of 100 µM EHNA (ADA 2) from total ADA activity.

ADA plays an important role in the purine salvage pathway (Green and Chan, 1993). This pathway (Fig. 1) is dependent upon a precise balance of ADA and adenosine kinases. Excessive adenosine deamination by ADA is thought to lead to increase in cellular production and excretion of purines (Green and Ishii, 1972). In contrast, excessive phosphorylation of adenosine disrupts pyrimidine synthesis at a late stage in biosynthesis, and the resulting accumulation of adenosine nucleotide may inhibit cellular proliferation as a consequence of secondary pyrimidine deficiency. In the normal cellular milieu, adenosine levels are

sufficiently low to present such an imbalance (Chan *et al*., 1973).

ADA activity has been reported to be high in lymphoid tissues and its inadequacy reported to cause immune deficiency (Gakis, 1996). Complete deficiency of ADA causes severe combined immunodeficiency that is inherited as an autosomal recessive trait. The patients presents in infancy with recurrent infections, lymphopenia, defective proliferative responses to mitogens, hypogammaglobulinemia, and an inability to mount specific antibody responses. Patients with a low level of residual ADA activity have a later onset of clinical disease owing to a slower and sometimes less complete loss of immune function. Unlike other primary immunodeficiencies caused by defects in lymphocyte signaling pathways, ADA deficiency is a systemic metabolic disorder. The enzymatic defect is expressed in all cells, and therefore the substrate for the enzyme, adenosine, accumulate in cells of all types. Immunodeficiency is thought to occur because immature lymphoid cells are particularly sensitive to the toxic effect of adenosine (Bollinger *et al*., 1996).

MATERIALS AND METHODS

SUBJECTS

The group of patients was from ESUT Teaching Hospital Enugu and Iyienu Mission Hospital Ogidi. Subjects were sixty (60) patients, 32 males and 28 females aged 18 to 60 years, divided into three group with regards to their serotypes of hepatitis virus infection viz: hepatitis B group (n=37), hepatitis C group (n=6). Sixty (60) apparently healthy subjects, 34 males and 26 females aged 20 to 62 years, served as the control group. Clinical diagnosis of patients was confirmed by serological tests, and all subjects were HIV negative.

Venous blood was collected from the subjects into plain vacuum tubes. After one hour, the blood samples were centrifuged at 3000 rpm for 5 minutes to obtain serum which was collected into plain sample bottles and labeled accordingly. Enzyme activity assay was carried out for ADA, AST, ALT, and ALP. Levels of total bilirubin and conjugated bilirubin were also estimated.

ESTIMATION OF ADENOSINE DEAMINASE ACTIVITY

Serum ADA activity was determine by a method described by Giusti and Galanti (1984), that was based on the Berthelot reaction because of its simplicity and ability to produce precise results.

PRINCIPLE

Adenosine deaminase catalyses the irreversible hydrolytic deamination of adenosine to produce inosine and ammonia. The ammonia formed reacts with phenol in the presence of hypochlorite to form an indophenol which in alkaline solution gives a blue coloured compound. The nitroprusside incorporated in the reagent acts as a catalyst, the intensity of the colour obtained is directly proportional to the amount of ammonia produced by the sample.

REAGENTS

i. Adenosine: 0.748 Mol/L

ii. Phosphate buffer, pH 7.4

iii. Phenol colour reagent:

iv. Alkaline hypochlorite reagent:

v. Ammonium sulphate standard: 75 µmol/L.

ANALYTICAL PROCEDURE

The tests were performed in batches of ten (10) samples daily alongside standards. Each test sample has a tube, the standard had a tube, and also the blank had a tube.

One (1) ml of ADA substrate was pipetted into a test tube for the test. This was followed by addition of 0.05ml of sample. After 60 minute incubation at 37^0C in a water bath, 5ml of phenol colour reagent was added, followed immediately by 5ml of the alkaline hypochlorite reagent. For the standard, 1ml ammonium sulphate was added to the test tube for standard. It was passed through colour development as for test. The contents of the tubes were mixed by inversion, and the tubes were then placed in a 37^0C water bath for 15 minutes. After the final incubation, the absorbance was read at 615nm spectrophotometrically after zeroing the instrument with distilled water.

One unit of ADA was defined as the amount of enzyme required to release 1µmol of ammonia/min from adenosine at standard assay conditions. ADA value was obtained by calculation using the standard, and results were expressed as international unit (U) of enzyme activity in serum.

CALCULATION

$$\mathbf{EA} = \frac{\mathbf{AT}}{\mathbf{AS}} \text{ X concentration of standard}$$

Where:

EA = Enzyme activity

AT = Absorbance reading of test

AS= Absorbance reading of standard

ESTIMATION OF ASPARTATE AMINOTRANSFERASE ACTIVITY

The serum aspartate aminotransferase (AST) activity was estimated by the method of Reitman and Frankel (1957), with kit assay system (Randox, United Kingdom).

ANALYTICAL PROCEDURE

Two test tubes were set up for each specimen to be analysed, one labeled T (test) and the other B (blank). 0.5ml of the buffer containing the substrate mixture was pipetted into each test tube, while 0.1ml of the

sample was added into tube T. The tube were mixed and incubated for exactly 30 minutes at 37^{0}C. At the end of the incubation time, 0.5ml of the 2, 4-dinitrophenylhydrazine reagent was pipetted into both tubes. 0.1 ml of the sample was added into tube B. Both tubes were mixed and allowed to stand for exactly 20 minutes at 20–25^{0}C. 5 ml of 0.4N NaOH was pipetted into the tubes, which were mixed and the absorbance of the sample was read after 5 minute against the sample blank spectrophotometrically at 546 nm. The activity of ALT in the sample was obtained from the table provided in the manufacturer's manual.

STATISTICAL ANALYSIS

Student's t test was used to compare result in both patients and controls (all results were reported as (mean ± SD), and Pearson correlation was used to test for association between parameters using a computer program named SPSS for windows release 16.0. A p-value < 0.05 was considered significant.

RESULTS

The result obtained in this study is presented in table 1- 3.

Table 1 shows Serum adenosine deaminase (ADA) activity and other liver function indices in patients with HBV, HCV and control group. The mean value of serum ADA activity in patients with HBV (22.47 ± 11.71U/L) and those with HCV (22.14 ± 8.55U/L) was significantly higher ($p<0.05$) than the mean values of serum ADA in the control group (10.99 ± 4.69U/L). The serum activity of AST (18.68 ± 11.87U/L), ALT (21.03 ± 12.38U/L), ALP (85.22 ± 39.13U/L) and TBIL levels (3.34 ± 4.78µmol/l) in patients with HBV and the mean values of AST (20.84 ± 13.36U/L), ALT (22.29 ± 11.92U/L) ALP (86.76 ± 67.03U/L) and TBIL (2.09 ± 1.34µmol/l) in those with HCV were significantly higher ($p<0.05$) than the corresponding values in the

control group; AST (9.35 ± 5.27U/L), ALT (5.50 ± 3.25U/L), ALP (43.65 ± 20.92U/L) and TBIL (1.48 ± 1.05 µmol/L). The CBIL level in patients with HBV (1.20 ± 3.62µmol/L) and those with HCV (0.53 ± 0.47µmol/l) were not significant (p>0.05) when compared with the control group (0.27 ± 0.40 µmol/L).

Table 2 shows Serum ADA activity and other liver function indices in patients co-infected with HBV and HCV. The Serum activity of ADA (19.83 ± 9.10U/L), AST (12.25 ± 7.89U/L), ALT (18.50 ± 4.95U/L), ALP (71.50 ± 9.31U/L), TBIL (1.89 ± 0.87U/L) in patients with HBV/HCV co-infection were significantly higher (p<0.05) that the corresponding values in the control group; ADA (10.99 ± 4.69U/L), AST (9.35 ± 5.27U/L), ALT (5.50 ± 3.25U/L), ALP (43.65 ± 20.92U/L), T_{BIL} (1.48 ± 1.05µmol/l). The C_{BIL} level in patients with HBV?HVC co-infection (0.16 ± 0.22µmol/l) was not significant (p>0.05) when compared with the control group (0.27 ± 0.40µmol/l).

Table 3 shows Serum ADA activity and other liver function indices in patients with HBV, HCV and HBV/HCV co-infection. There was no significant difference (p>0.05) in ADA activity in patients with HBV (22.47 ± 11.71U/L), HCV (22.04 ± 8.55U/L) and HBV/HCV co-infection (19.83 ± 9.10U/L) and other liver function indices.

TABLE 1: SERUM ADA ACTIVITYAND OTHER LIVER FUNCTION INDICES IN PATIENTS WITH VIRAL HEPATITIS AND CONTROL GROUP

	HBV PATIENTS (n – 37	HCV PATIENTS (n – 17)	CON (n –
ADA (U/L)	22.11 ± 10.42	22.04 ± 8.55	10.99
AST (U/L)	18.68 ± 11.87	20.82 ± 13.36	9.35
ALT (U/L)	21.03 ± 12.38	22.29 ± 11.92	5.50
ALP (U/L)	85.22 ± 39.13	86.76 ± 67.03	43.65
TBIL(µmol/L)	3.34 ± 4.78	2.09 ± 1.34	1.48
CBIL (µmol/L)	1.20 ± 3.61	0.53 ± 0.47	0.27

Results are Mean ± SD; p < 0 > 0.05

KEY: n = Number of samples

ADA = Adenosine deaminase

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

ALP = Alkaline phosphatase

TBIL = Total bilirubin

CBIL = Conjugated bilirubin

TABLE 2: SERUM ADA ACTIVITYAND OTHER LIVER FUNCTION INDICES IN PATIENTS CO-INFECTED WITH HBV AND HCV

	HBV/HCV CO-INFECTED PATIENTS(n-6)	CONTROL(n-60)	*p* value
ADA (U/L)	**19.83 ± 9.10**	**10.99 ± 4.69**	**<0.05**
AST (U/L)	**12.24 ± 7.89**	**9.35 ± 5.27**	**<0.05**
ALT (U/L)	**16.50 ± 6.81**	**5.50 ± 3.25**	**<0.05**
ALP (U/L)	**74.00 ± 10.99**	**43.65 ± 20.92**	**<0.05**
TBIL(µmol/L)	**1.86 ± 0.87**	**1.48 ± 1.05**	**<0.05**
CBIL (µmol/L)	**0.16 ± 0.22**	**0.27 ± 1.69**	**>0.05**

Results are Mean ± SD; p < 0 > 0.05

KEY: n = Number of samples

ADA = Adenosine deaminase

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

ALP = Alkaline phosphatase

TBIL = Total bilirubin

CBIL = Conjugated bilirubin

TABLE 3: SERUM ADA ACTIVITY AND OTHER LIVER FUNCTION INDICES PATIENTS WITH HBV, HCV AND HBV/HCV CO-INFECTION

	HBV PATIENTS (n-37)	HCV PATIENTS (n-17)	HBV/HCV CO-INFECTED PATIENTS

			(n-6)
ADA (U/L)	**22.47 ± 11.71**	**22.04 ± 8.55**	**19.83 ± 9.10**
AST (U/L)	**18.68 ± 11.87**	**20.82 ± 13.36**	**12.24 ± 7.89**
ALT (U/L)	**21.03 ± 12.38**	**22.29 ± 11.92**	**16.50 ± 6.81**
ALP (U/L)	**85.22 ± 29.13**	**86.76 ± 67.03**	**74.00 ± 10.99**
TBIL(µmol/L)	**3.34 ± 4.78**	**2.09 ± 1.34**	**1.86 ± 0.87**
CBIL (µmol/L)	**0.16 ± 0.22**	**0.27 ± 0.40**	**0.16 ± 0.22**

Results are Mean ± SD; p < 0 > 0.05

KEY: n = Number of samples

ADA = Adenosine deaminase

AST = Aspartate aminotransferase

ALT = Alanine aminotransferase

ALP = Alkaline phosphatase

TBIL = Total bilirubin

CBIL = Conjugated bilirubin

KEY: r= Pearson correlation coefficient

DISCUSSION

In this study, serum activities of ADA in viral hepatitis patients were significantly higher than those in the control group (p< 0.05). So were the activities of AST, ALT, ALP, and levels of TBIL were significantly higher than corresponding values in the control (p<0.05). Values of CBIL were not significant when compared with corresponding values in the control group (p>0.05). There was no significant difference between the serum activities of ADA and other liver function indices in HBV infected patients, HCV infected patients, and patients co-infected with HBV and HCV (p>0.05). According to gender distribution (sex), no statistically significant difference in serum ADA activity and other liver function indices in the patient and the control groups was found (p> 0.05). This result agrees with earlier result (Kalkan *et al*.,

1999 Vasudha *et al*., 2006; Kaya *et al.,* 2007).

Vasudha *et al.* (2006) reported that several ADA activities remain steady from 31-60 years, with a steep rise in activity above 60 years of age. They observed that normally from 0-30 years, there is only a slight increase in the activity of serum ADA. Also, in the report of Kalkan *et al.* (1999), they found no difference in the ADA activities of acute and chronic hepatitis B cases. For these reason, in the present study, serum activity of ADA in these patients were evaluated without taking the chronicity of the disease and the age of the patients into consideration, since the mean age of all the subjects in this study was above 34 years.

There was no significant correlation between serum ADA activity with levels of ALP ($p>0.05$). On the contrary, a significant positive correlation was found between ADA activity and those of AST, ALT, and levels 0f bilirubin ($p< 0.05$). This agrees with the report of Kaya *et al*. (2007), which suggest that serum ADA activities are associated more with the activities of serum transaminases than viral load in HBV-and HCV- infected patients. The positive correlation of serum ADA activity with levels of bilirubin also agrees with the work of Pratibha *et al*. (2004) who reported a significant positive correlation between ADA and bilirubin. The increase in bilirubin levels, though small, could also be looked upon as a protective mechanism which the liver has evolved in other to combat oxidative stress so as to maintain a normal function.

CONCLUSION

Increase in serum ADA activity corresponds to those of ALT, AST and levels of bilirubin in HBV, HCV and

HBV/HCV co-infected patients. This increase, which is the same in both males and females, may be dependent on and reflect the increase in phagocytic activity of macrophages and maturation of T-lymphocytes, and may be valuable in monitoring patients with viral hepatitis.

RECOMMENDATION

ADA is a useful index in diagnosis and monitoring of patients with viral hepatitis. I recommend that its estimation be added in our routine liver function tests.

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