

Variations In Ada Levels In Various Body Fluids- An Original Study

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Abstract:

Aim: The aim of our study was to determine the frequency of various types of basal cell carcinomas encountered in our practice, to delineate the spectrum in our setup and to determine the different histological patterns, anatomical location, site predilection, and age and sex incidence.

I. Introduction

Adenosine deaminase levels : enzyme of purine metabolism that deaminates adenosine and deoxy adenosine to inosine and 2 deoxy inosine respectively. ADA deficiency leads to SCID. The abnormality of this enzyme is associated with hereditary non spherocytic hemolytic anaemia. It is only RBC enzymopathy that is inherited as an autosomal recessive disorder. (Tietz Biochemistry) More ADA activity is present in the cytoplasm of T cells than in the cytoplasm of B cells. ADA activity is usually elevated in early HIV infection as a result of stimulation.

(Williams Haematology)

Cut Off Value

CSF -10

Serum -18

Pleural Fluid - 33 U/L

ADA Sample Is Stable For 7 Days At 2-8 Degree Centigrade.

Interferences: Hemolysis Interferences With ADA Level Lipaemia And Bilirubin Don't Interfere.

ADA can be elevated in liver patients (reference); Kobayashi F, Ikeda T, Marumo F, Sato C. Adenosine deaminase isoenzymes in liver disease. Am J Gastroenterol 1993;88:226-71.

Fluid Type	CSF	Ascitic Fluid	Pericardial Fluid	Synovial	ECF Fluid Pt	Serum	Pus	Liver Abscess	Parotid
Male			28			9			
Female			33		465	11			
Total			61		1152	20			
Cut Off Value									
More Than									
Less Than									

Total ADA 5418 samples

Male -3839

Female -1589

ICD FLUID -6 ADA-CSF-1450 Ascitic Fluid-700 Peritoneal Fluid-14 Synovial -10 Serum-20 Pus-8 All R

More Than 200

Liver Abscess- 2

Parotid -1 Capd Fluid-3

More than 36-3839 CSF More than 36-50

Liver abscess

More Than 500-2 Capd Fluid Less Than 2

ADA

Total--3839 Male-2245 .More Than 36 Is 1152.

Pericardial- 61 Male -28 Fema-33

Less Tahn 1.00 Value-36
Less Tahn Value-36-----1081 Normal
More Tahn Value-36----1152
Csf Ada Noraml Is 0-6
In Which One Is More Than 6
Peritonea Fluid Total -11 Out Of Which 4 Have More Than 36 Rest Normal.
Synovial Fluid Is 9 Out Of This 6 Is More Than 36.
Serum Ada -Total Is 20
9 Male 11 Female
Normal Reporting Is More Than 60 Detected .40-60 Indeterminate ..
More Than 60 Is 3 Only
Omkar Is 23 Samples .
Total Plueral Fluid Is 1152 . Out Of This 461 Female
Ascitic Is 698
Cyst Aspiration Fluid# (Fluid Vol - 40 MI) -1 Value Is 35
Age Less Than 10yrs Is 40 . Age 0 Is 43..
14 Years Is 3 5
Capd Fluid Range Not Defined Toatl -3 Value 4,8,Less Than 1.

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Exclusion Criteria: Patients undergoing treatment for any thyroid disorders, patients taking lipid-lowering drugs, patients with diabetes, patients with malignancy, and pregnant women were excluded.

Collection and Analysis of Blood Samples: Informed consent was obtained from all subjects for participating in the study. Blood samples were collected by venipuncture using an aseptic technique. The serum separated from the samples was analyzed for following biochemical parameters.

Blood samples were analyzed for: Blood glucose fasting by using enzymatic glucose oxidase–horseradish peroxidase-based end-point method. HbA1c was determined by ion-exchange resin method.

Serum separated from the samples was analyzed for: Thyroid function tests (T3, T4, and TSH) by RIA method. Fructosamine test was conducted using nitroblue tetrazolium method.

II. Materials And Methods

Activity of ADA was determined in 60 children of age range 6 months to 11 years admitted to department of pediatrics at the Postgraduate Institute of Medical Education and Research, Chandigarh, India during 1997-98. Clinical and laboratory evidence of meningitis/ meningoencephalitis was taken as inclusion criteria. Enzyme activity was measured in CSF of four groups of patients i.e., TBM, PTM, AM and PM. These four groups were characterised as given below. Pyogenic meningitis (PM) group In this group, CSF of patients showing organisms in gram stained smear or culture or presence of bacterial antigen on latex agglutination was taken Indian Journal of Clinical Biochemistry, 2004, 19 (2) 5-9 Indian Journal of Clinical Biochemistry, 2004 6 as diagnostic criteria. In the absence of organisms CSF showed pleocytosis of more than 100 cells/ mm³ predominantly polymorphs, sugar less than half of blood sugar and protein more than 60 mg % . This was taken as inclusion criteria. Partially treated pyomeningitis (PTM) group This group consisted of patients in whom their CSF showed presence of organisms on gram stained smear or culture or presence of bacterial antigen. In the absence of organism CSF showed pleocytosis of more than 100 cells /mm³, sugar less than half of blood sugar, protein more than 60 mg % and who had received I.V. antibiotics for pyogenic meningitis for more than 48 hours before coming to hospital. Aseptic meningitis (AM) group This group consisted of patients whose CSF showed absence of organisms on gram stain or culture and CSF pleocytosis with more than 10 cells/mm³,

predominantly lymphocytes and sugar more than 2/3 of blood sugar value. Tuberculous meningitis (TBM) group In this group the patients had two or more of the

following features on history; fever for > two weeks, contact with an adult with tuberculosis, positive Mantoux test. This group had CSF with absolute lymphocyte counts > 50 cells/mm³, protein more than 60 mg % and sugar less than 2/3 of blood sugar, chest X-ray showing skiagram suggestive of pulmonary TB, isolation of AFB from any site, CT scan showing evidence of chronic meningitis like hydrocephalus, basal exudates, infarcts, tuberculomas and histological evidence of tuberculosis. Activity of ADA was assayed according to the method of Guisti (9). Two ml of CSF sample was collected in a sterile bottle at the time of admission. Patients in whom the lumbar puncture was traumatic were excluded from the study. The samples obtained were centrifuged at 2000 g for 10 min and the supernatant stored at -20°C until estimation. One unit of activity represented the deamination of one micromole of adenosine /min at 37°C and was expressed as U/L. Statistical Analysis The means ± standard error (SE) of ADA values was calculated for each group of subjects. Wilcoxon's rank sum test was used to determine

statistical significance. Pearson's formula was used to calculate the coefficient of correlation (r-value).

III. Reagent

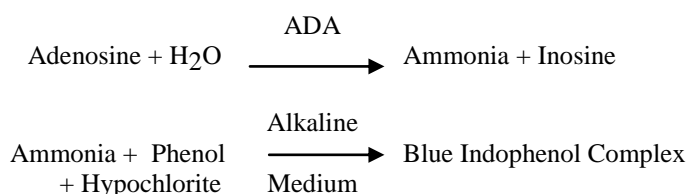
Microexpress Ada-Mtb Is A Reagent For Laboratory Use Only.

Ada-Mtb Comprises Of:

- a) Ada-Mtb Reagent (L1) – Buffer Reagent, Ready To Use
- B) Ada-Mtb Reagent (L2) – Adenosine Reagent, Ready To Use
- C) Ada-Mtb Reagent (L3) – Phenol Reagent
- D) Ada-Mtb Reagent (L4) – Hypochlorite Reagent
- E) Ada-Mtb Standard (S) – Ada Standard, Ready To Use

Principle

Adenosine Deaminase hydrolyses adenosine to ammonia and inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form a blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured indophenol complex formed is directly proportional to the amount of ADA present in the sample.



Reference Values

Serum, Plasma, Pleural, Pericardial Ascitic Fluids	Normal	< 30 U/L
	Suspect	30 U/L to 40 U/L
	Strong Suspect	> 40 U/L to 60 U/L
	Positive	> 60 U/L
CSF	Normal	< 10 U/L
	Positive	> 10 U/L

It is recommended that each laboratory establish its own normal range representing its patient population.

Storage And Stability

(1) Store the ADA-MTB kit at 2-8°C, away from light. (2) Stability of the ADA-MTB kit is as per the expiry date mentioned on the label.

Note

(1) It is important that kit components from the same lot are used for achieving accurate and reproducible results. Do not intermix reagents from different lots. (2) The sequence of addition of reagents should be followed meticulously for achieving accurate results.

Additional Material Required

Test tubes, test tube stand, waterbath/incubator (37°C), distilled or deionised water, variable volume pipettes, spectrophotometer with filter at 570-630 nm (Hg 578 or 623 nm) at 37°C or colorimeter with yellow or red filter, stopwatch.

Reagent Preparation

Reagents L1, L2 and standard are ready to use. **Adenosine Reagent (L2) may form crystals at 2-8⁰C. Dissolve the same by gently warming the reagent for some time before use.** Both the Phenol Reagent (L3) & Hypochlorite Reagent (L4) need to be diluted 1: 5 with distilled water before use (1 part of reagent + 4 parts of distilled water). The Working Phenol Reagent and Working Hypochlorite Reagent are stable for at least 6 months when stored at 2-8⁰C in tightly closed bottles.

Specimen Collection And Preparation

Collect specimen prior to use of antimicrobial agent. Wherever possible, indicate clearly that patient is on antitubercular drugs.

CSF: Collect as much as possible in a syringe, clean skin with alcohol before aspirating specimen.

Body fluids: Disinfect the site and collect specimen with aseptic precautions.

Serum, Plasma: No special preparation of the patient is required prior to sample collection by approved techniques. It is recommended to use fresh sample specimen for testing. Do not use haemolyzed, contaminated or turbid sample specimens. Fresh EDTA, citrate, heparinised or oxalate anticoagulated plasma specimens are suitable for performing the test.

Ada is reported to be stable in serum for 3 days at 2-8⁰C and in biological fluids for 2 days at 2-8⁰C, as after this, ammonia may be released in the samples even without any microbial contamination.

Test Procedure

1. Bring all reagents and samples to room temperature before use.
2. Prepare the Working Phenol Reagent and Working Hypochlorite Reagent.
3. Set the spectrophotometer filter at 570-630 nm (Hg 578 or 623 nm) at 37⁰C.
4. Pipette into clean dry test tubes labeled Blank (B), Standard (S), Sample Blank (SB) and Test (T) as follows:

Addition Sequence	B (ml)	S (ml)	SB (ml)	T (ml)
Buffer Reagent	0.20	0.20	-	-
Adenosine Reagent	-	-	0.20	0.20
Deionised water	0.02	-	-	-
Standard	-	0.02	-	-
Sample	-	-	-	0.02

5. Mix well and incubate at 37⁰C for exactly 60 minutes, and then add the following:

Working Phenol Reagent	1.00	1.00	1.00	1.00
Sample	-	-	0.02	-
Working Hypochlorite Reagent	1.00	1.00	1.00	1.00

6. Mix well and incubate at 37⁰C for 15 minutes or at R.T. for 30 minutes.
7. Measure the absorbance of the Blank (Abs. B), Standard (Abs. S), Sample Blank (Abs. SB) and Test (Abs. T) against distilled water.

Calculations

$$\text{Total ADA activity in U/L} = \frac{\text{Abs. T} - \text{Abs. SB}}{\text{Abs. S} - \text{Abs. B}} \times 50$$

Linearity

The procedure is linear upto 150 U/L. If values exceed this limit dilute the sample with deionised water and repeat the assay. Calculate the value using the appropriate dilution factor.

Remarks

1. One unit of ADA activity releases three nanomoles of ammonia in the reaction in 1 hour at 37⁰C.
2. Patients with hyperamoniemia, kidney disorders and hepatitis can present high levels of ADA values. Patients with chronic malnutrition or HIV can present low levels of ADA values.
3. Using a cut off level of 60 units/L of ADA, values has been reported to show the Specificity and the Sensitivity of the test as above 90% for the MTB infection.
4. Below 60U/L of ADA, the serum ADA specificity and sensitivity is lower and should be interpreted in the light of other tests for confirmation of *Mycobacterium tuberculosis* infection.

IV. Summary

Tuberculosis occurs worldwide and is rampant in many countries. Though curable, its infection is on the rise. The most specific test is the positive bacterial culture of a patient's sputum sample. This is cumbersome and time consuming. X-rays, smears for AFB and Tuberculin tests though comparatively rapid are not conclusive. Adenosine Deaminase (ADA) is an enzyme widely distributed in mammalian tissues, particularly in T Lymphocytes. Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same. Though ADA is also increased in various infectious diseases like Infectious Mononucleosis, Typhoid, Viral Hepatitis, initial stages of HIV, and in cases of malignant tumours, the same can be ruled out clinically.

Bibliography

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