ORIGINAL RESEARCH PAPER

Biochemistry

SERUM ADENOSINE DEAMINASE AS MARKER OF HEMOLYSIS IN ADULTS WITH HEMOLYTIC ANEMIA WITH PULMONARY HYPERTENSION

KEY WORDS: ADA HEMOLYTIC ANEMIA

Dr.Madhav Shridharrao Kadam Assistant Professor Biochemitry Dr.S. C.Gmc Nanded

Mis Rupali Wankhede Assistant Professor Biochemistry Dr.R.R. Kambe Dental College and Hospital Akola

ABSTRACT

Tissue specific increase in normal adenosine deaminase is associated with hereditary hemolytic anemia.to validate the relevance of ada as marker of hemolysis in adults, ada was estimated by colorimetric method of glusti and gulani based on bertholet reactions

INTRODUCTION

Adenosine deaminase is a purine catabolic enzyme that catalyses the irreversible deamination of adenosine to inosine and 2 deoxyadenosine to 2 deoxyinosine . abnormality of adenosine deaminase have been reported in association with immune dysfuction, acute leukaemia and hereditary hemolytic anaemia. ADA gene expressed in all tissues and thus may be categorized as a housekeeping gene. However the level of expression varies by more than 1000 fold in different tissues and development states with highest level in fibroblast are normal. metabolic study with patients erythrocytes show that low ATP concentration in these cells is due to a diminished synthesis of adenylic nucleotide from adenosine and to an excessive catabolism of AMP. The resultant depletion of ATP deprives erythrocytes of their major energy sources and leads to loss of membrane integrity and premature destruction.

The specific molecular defect underlying the tissue enzymes over expression has not been elucidated. From linkage analysis it has been determined that the mutation causing RBCS specific ADA overproduction lies within or near ADA locus. Furthermore recent advances indicates that ADA is strongly elevated in the vascular complications seen in hemolytic anemia most notable of which is pulmonary hypertension.

MATERIAL METHODS

The patient population consisted of 100 adults in age group of 40 to 50 yr. these hundred children divided into two groups. First group consist of 50 diagnosed cases of hemolytic anemia adults. Second group consist of 50 adults who were diagnosed as anemia due to causes other than hemolytic anemia. adults of first and second groups were admitted as in patients deparment of medicin of dr.s c. gmc vishnupuri nanded of 40 to 50 age sex matched healthy controls. Informed consent was taken from patients and their relatives and research protocol was approved by the institution ethical committee. eligiblity criteria included adults bet the age group of 40 to 50 yrs who were diagnosed with hemolytic anemia (including hemoglobinopathies, erythroenzymopathy) all patients provided medical histories blood samples underwent physical examination and baseline laboratory and radiological investigations. All adults diagnosed with pernicious anemia, rheumatoid arthritis tuberculosis, immunological liver and renal disorders and concomitant comorbidity were excluded from study.

METHOD

Venous blood 3.5ml was collected and transferred into sterile EDTA/Heparin tubes. The tubes were gently rotated to mix the content and centrifuged at 2000x for 20 min at 4 centrigate and the supernantant was discarded. Pellet containinh RBCS were washed thrice with ice cold 0.85%$ for 10 min at 4 centrigate. Final pellet was taken up in 4ml chilled water, left in cold for 1 hour for hemolysis and then centrifuged at 200 for 20 mins. The supernatant thus obtained was used for analysis. ADA estimation was done by colorimeteric method of glusti and gulani method based on bertholet reaction

STATISTICAL ANALYSIS

The comparison of medians was done by KruskalWallis test and Mann-Whitney test. Correlation was done using Spearman correlations. Results were considered significant if p value was less than or equal to 0.05. Statistical analysis was done using SPSS 11.5 software.

DISSCUSION

Disorders of red blood cell enzymes membranes and haemoglobin casuses hereditary hemolytic anemias. Kinetic and electrophoretic studies have shown that most of erthroenzymopathies are due to overproduction of a mutant enzymes caused by point mutations an exception is hemolytic anemia secondary to increased adenosine deaminase activity. Red cell ADA activity increases in affected individual. Basic abnormality appears to result from overproduction of catalytically and immunologically normal enzymes due to abnormal translational efficiency. Such an increase could result either from overproduction of the enzyme or from increased stability of the protein in erythrocytes. Hirschhorn has shown that ADA activity in normal RBCs is highly stable with a half-life of about 231days 16. Since the average RBC life span is 120 days, it is impossible to attribute a 70-to100-fold increase in ADA activity to a structural gene mutation that increases the protein stability1. The degree of secondary structure of the 5' noncoding region of the mRNA appears to be inversely proportional to the translational efficiency of the mRNA. The 5' non-coding region of ADA mRNA is comprised of 80% guanosine and cytosine residues 17 with considerable potential for formation of stable secondary structures. Mutations in this region and consequent loss of secondary structure might result in increased ribosomal binding to and /or scanning of the mRNA. Point mutations flanking the initiation codon have also been shown to alter translational efficiency 7,17,18. The mechanism underlying the tissue specificity of this defect is not yet clear. Inhibitors of translation have been demonstrated in erythroid cells19, and one potential mechanism for specific increased translational efficiency of ADA mRNA in red cell is a mutation that prevents the binding of an inhibitor. It is also possible that tissue specific splicing of a primary RNA transcript may give rise to minor differences in the 5' non-coding regions of ADA mRNAs that thus affect translational efficiency1.

Recent responses that may reduce hemolysis-induced vasculopathy and the risk of PH. Hypoxia is the strongest stimulus for ADO synthesis, and this increased ADO production counteracts some of the tissue/vascular injury caused by hypoxia itself. Unfortunately, under hypoxic conditions (anemia, vasoconstriction, and vaso-occlusion) in HA-PH, this "ADO negative-feedback" is abolished and the vascular protective

effects of ADO are severely diminished by ADA released from injured erythrocytes 13,14. Our study suggests that both ADA and LDH may be useful biomarkers in children with both types of anemia as compared to controls (Table 1) but of the two erythrocytic enzymes only mean ADA was found to be significantly raised in hemolytic anaemia group when compared to anemia of other causes (p<0.001). These data confirm the marked

RESULTS

The medians of both Ada levels higher in adults with both types of anemia as compared to controls (Table 1) but of the two erythrocytic enzymes only ADA was found to be significantly raised in hemolytic anemia group when compared to anemia of other causes (p < 0.001)

	GROUP 1	GROUP 2	CONTROL GROUP	P VALUE
HEMOGLOBIN(%)	6	8	13	< 0.001
RETICULOCYTE COUNT(%)	9	6	0.7	<0.001
ADA LEVELS(IU)	4.15	1.6	1	<0.001

(Figures 1 and 2). These data confirm the marked elevation of erythrocyte ADA in hemolytic anemia. Correlation analysis was performed between ADA and Hb% and reticulocyte count. ADA correlated significantly with Hb% but only ADA and not Recent advances indicate that both ADA and LDH are strongly elevated in the vascular complications seen in hemolytic anemias, the most notable of which is pulmonary hypertension. Hemolysis-associated pulmonary hypertension (HA-PH) is a serious clinical complication of various hemolytic disorders, and pulmonary hypertension (PH) is considered the greatest risk factor for death in patients with a hemolytic disorder. It is now well established that hemolysis causes the release of soluble hemoglobin and arginase from injured erythrocytes into plasma. This leads to nitric oxide (NO) deficiency, oxidative stress and a state of endothelial dysfunction that is associated with clinical development of pH9. The red cell membrane normally serves as a physical and diffusion barrier that segregates erythrocyte proteins from plasma and endothelium. Intravascular hemolysis disrupts this protective compartmentalization. This allows 2 sets of pathological biochemical reactions to occur. The first involves the stoichiometric inactivation of NO by cell free plasma GROUP 1GROUP 2 CONTROL GROUPP VALUEHEMOGLOBIN (%) 6813< 0.001RETICULOCYTE COUNT(%) 960.7<0.001ADA LEVELS(IU) 4.151.61<0.001hemoglobin with consequent impairment of NO-dependent blood flow. The second involves the release of erythrocyte arginase, which converts plasma L-arginine to ornithine, resulting in depletion of plasma L- arginine, the required substrate for NO production by NO synthase, with associated pulmonary hypertension. Newer evidences indicate that in addition to the NO-arginase pathway, thE adenosine pathway plays a significant role in HAPH and that modulation of this pathway may offer protective/therapeutic effects in HAPH13.Preliminary data suggest that in HA-PH adenosine deaminase (ADA) is released from injured erythrocytes into plasma and that metabolic conversion of adenosine (ADO) to inosine by ADA reduces extracellular ADO levels. Adenosine, mainly via activation of adenosine A(2A) receptors, mediates a number of biological LDH corelevation of erythrocyte ADA in hemolytic anemia. Correlation analysis was performed between these two erythrocytic enzymes and Hb% and reticulocyte count. ADA is correlated significantly with Hb% but only ADA correlated significantly with reticulocyte count also (p <0.00, here difference in r value was significant with p value 0.003, Z-score 2.274). These data document that although ADA could be marker of hemolysis

REFERENCE

1. Chottiner EG, Cloft HJ, Tartaglia AP and Mitchell BS. Elevated Adenosine Deaminase Activity and Hereditary Hemolytic Anemia. J Clin Invest. 1987;79:1001-5.
2. Chottiner EG, Ginsburg D, Tartaglia AP and Mitchell BS. Erythrocyte Adenosine Deaminase Overproduction in Hereditary Hemolytic Anemia. Blood. 1989;74:448-53.
3. Aronow B, Lattier D, Silbiger R, Dusing M, Hutton J, Jones G et al. Evidence for a complex regulatory array in the first intron of the human adenosine deaminase gene. Genes Dev. 1989;3:1384-1400.
4. Chinsky JM, Maa M-C, Ramamurthy V and Kellems RE. Adenosine Deaminase Gene Expression Tissue-Dependent Regulation of Transcriptional Elongation. J Biol Chem. 1989;264(24):14561-5.
5. Perignon JL, Hamet M, Buc HA, Cartier PH and Derycke M. Biochemical study of a case of hemolytic anemia with increased (85 fold) red cell adenosine deaminase. Clin Chim Acta. 1982;124(2):205-12.
6. Kanno H, Tani K, Fujii H, Iguchi-Ariga SM, Ariga H, Kozaki T et al. Adenosine deaminase (ADA) overproduction associated with congenital hemolytic anemia: case report and molecular analysis. Jpn J Exp Med. 1988;58(1):1-8.
7. Fujii H and Miwa S. Pathophysiology and laboratory tests of hemolytic tests of hemolytic anemia: with special reference to erythroenzymopathies. Rinsho Byori. 1989;37:1331-43.
8. Aster JC. Red Blood Cell and Bleeding Disorders. In:Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran Pathologic Basis of Disease, 7th edn. Philadelphia: W. B. Saunders. 2005:619-59.
9. Kato GJ, McGowan V, Machado RF, Little JA, Taylor VI J, Claudia R et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension and death in patients with sickle cell disease. Blood. 2006;107(6):2279-85.
10. O'Driscoll S, Height SE, Dick MC and Rees DC. Serum lactate dehydrogenase activity as a biomarker in children with sickle cell disease. Br J Haematol. 2008;140:206-9.
11. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, Minter K et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med. 2004;350:886-95.
12. Kato GJ, Martyr S, Blackwelder WC, Nichols JS, Coles WA, Hunter LA et al. Levels of soluble endothelium derived adhesion molecules in patients with sickle cell disease are associated with pulmonary hypertension, organ dysfunction and mortality. Br J Haematol. 2005;130:943-53.
13. Van Linden A and Eltzschig HK. Role of pulmonary adenosine during hypoxia: extracellular generation, signaling and metabolism by surface adenosine deaminase/CD26. Expert Opin Biol Ther. 2007;7(9):1437-47.
14. Tofovic SP, Jackson EK and Rafikova O. Adenosine deaminase-adenosine pathway in hemolysis-associated pulmonary hypertension. Med Hypotheses. 2009:72(6):713-9.
15. Giusti G.Adenosine Deaminase. In: Bergmeyer HU, editor. Methods of enzyme analysis. New York: Academic Press. 1974:1092-9.
16. Hirschhorn R, Roegner V, Jenkins T, Seaman C, Piomelli S and Borkowsky W. Erythrocyte adenosine deaminase deficiency without immunodeficiency. J Clin Invest. 1979;56:1130-9.
17. Pelletier J and Sonenberg N. Insertion mutagenesis to increase secondary structure within the 5' noncoding region of eukaryotic mRNA reduces translational efficiency. Cell. 1985;