AFRICAN JOURNAL OF MEDICINE
and medical sciences

VOLUME 21, NUMBER 2, DECEMBER 1992

EDITOR: B.O. ONADEKO
ASSISTANT EDITORS:
B.O. OSOTIMEHIN and A.O. UWAIFO

SPECTRUM BOOKS LIMITED
Ibadan • Owerri • Kaduna • Lagos

ISSN 1116–4077

Afr. J. Med. med. Sci. (1992) 21, 13-17

Alpha-hydroxybutyrate dehydrogenase and the diagnosis of painful crisis in sickle cell anaemia

G.I. AKENZUA, J.C. IHONGBE and H.N. ASEMOTA+

Institute of Child Health College of Medical Sciences, and +Department of Biochemistry University of Benin

Summary

To assess the value of α-hydroxybutyrate dehydrogenase (α-HBDH) in the diagnosis of painful crisis (PC) of sickle cell anaemia (SCA), we studied plasma enzyme levels in 55 children with HbSS and 21 control subjects with haemoglobin genotype AA.

In 21 chidren with SCA, mean plasma α-HBDH was 373.8 ± 113.5 μ/l during PC and during steady state in 34 children, it was 341.2 ± 103.4 μ/l. These values were significantly higher than that of 128 ± 19.5 μ/l obtained in control subjects.

However, the difference between mean plasma α-HBDH levels in SCA children in PC and in steady state was 32.6 μ/l, $t = 1.095$; $P < 0.2$. There was no correlation between α-HBDH levels and reticulocyte counts ($r = 0.0856$; $t = 0.4565$; $0.7<P<0.6$).

The high levels of α-HBDH in patients with SCA is probably due to chronic haemolysis and not marrow infarction. Therefore, α-HBDH is of doubtful value in the diagnosis of painful crisis.

Résumé

Afin de déterminer la valeur de α-hydroxybutyrate dehydrogenase (α-HBDH) dans la diagnose des crises de douleur, nous avons étudié les niveaux enzymatiques du plasma chez 55 enfant souffrant de l'anémie de l'hématie falciforme (SCA) et 21 sujets de controle ayant le genotype AA d'hémoglobine.

Chez 21 enfants ayant le plasma moyen de SCA, α- HBDH etait 373.8 ± 113.5 μ/l pendant PC, et chez 34 enfants 341.2 ± 103.4 μ/l pendant l'etat fixe. Ces valeurs etaient nettement superieures a 128 ± 19.5 μ/l obtenu chez des sujets de controle.

Néanmoins, la différence entre l'échantillon des niveaux du plasma moyen α-HBDH chez les enfants (SCA) dans PC at l'etat fixe etait 32.6 μ/l, $t = 1.095$; $P<0.2$. Il n'y avait pas de correlation entre les niveaux de α-HBDH et les numérations reticulocytes ($r = 0.0856$; $t = 0.4565$; $0.7<P<0.6$).

Les niveaux élevés du α-HBDH chez les patients souffrant de la SCA sont probablément dus a l'hémolyse chronique et non pas a l'infarctus de la moelle. Donc, α-HBDH est d'une valeur douteuse dans la diagnose de crises de douleur.

Correspondence: Dr Gregory I. Akenzua Institute of Child Health, College of Medical Sciences, University of Benin, Benin City.

Introduction

Painful crisis (PC) is a characteristic feature of sickle cell anaemia (SCA) and in children it is the commonest cause of morbidity associated with sickle cell disease[1]. Several factors are known to precipitate PC including cold environmental temperature[2], infection[3], pyrexia[4], dehydration and acidosis[5]; but the exact role of these factors in the genesis of PC is not clearly understood. In African children with SCA, malaria infection is the commonest cause of PC[6].

Several changes have been observed in the blood during PC including leukocytosis[7], impaired neutrophil migration[8], low leukocyte alkaline phosphatase score[9], transient thrombocytosis[10] and elevated plasma haemoglobin levels[11]. However, these changes are not specific and they cannot be used for laboratory diagnosis of PC. Thus, the objective diagnosis of PC remains a challenge to physicians caring for patients with SCA.

Neely *et al*, in 1969[12] observed changes in serum lactic dehydrogenase (LDH) activity and isoenzymes pattern during PC; and others[13] confirming their observations have suggested that α-hydroxybutyric acid dehydrogenase (α-HBDH), iso-enzymes 1 and 2 LDH may be used for objective diagnosis of infarctive crisis. It was our purpose in this study to find out whether α-HBDH is of value in the diagnosis of painful crisis in Nigerian children with SCA. Therefore, we measured plasma α-HBDH levels in SCA children in steady state and also in PC and we compared them with individuals with normal haemoglobin genotype AA.

Materials and Methods

Sickle cell patients

The subjects were 55 children (aged 1-16 years) attending the paediatric haematology clinic at the

University of Benin Teaching Hospital (UBTH), in whom diagnosis of SCA was made according to standard criteria[14]. They were seen regularly in the clinic at intervals of 6-8 weeks and they were encouraged to come to the clinic for any acute illness occurring in the interval between clinic visits. Everyone of them was on routine folate supplementation (folic acid 1.5mg daily) and malaria prophylaxis (pyrimethamine 12.5-25mg weekly). Thirty-four of them were studied steady state and 21 during painful crisis. Steady state was defined as the condition of a known SCA patient who has had no acute illness for six weeks and who after a careful physical examination shows no obvious change in his usual clinical and haematologic features including degree of jaundice, size of spleen or liver, haematocrit, reticulocyte count and serum bilirubin level. Painful crisis was defined as pain in the chest, abdomen or limbs with or without fever that cannot be explained on any basis other than sickle cell disease[15].

Control subjects

Control subjects were 21 healthy individuals with haemoglobin genotype AA comprising 8 adult laboratory workers and 13 children (aged 2-15 years) who had venesection for various reasons. Nine had blood taken as part of routine medical examination (Haemoglobin electrophoresis) for admission to secondary school and 4 as part of pre-operative haematologic work up for surgical procedures including, two herniorrhaphies, one elective appendicectomy and one circumcision.

Four millilitres of venous blood was obtained from the cubital fossa with a plain plastic syringe and 3mls for enzyme assay was transferred to a plastic tube containing heparin 0.2μ/ml. The remaining sample for blood counts was placed in a sequestrene bottle containing appropriate amount of EDTA. The sample for enzyme assay was centrifuged at 1500*g* for 10 minutes at room temperature (average 25°C) and the platelet poor plasma obtained was frozen within 30 minutes of blood collection and stored at –70°C until it was required for assay.

Laboratory methods

Venous haematocrit and reticulocyte counts were determined according to standard haematologic techniques[16] using EDTA anticoagulated blood.

Enzyme assay

All assays were performed within two weeks of blood collection. Frozen samples were thawed at 30°C for 5 minutes and 0.1ml of platelet poor plasma (PPP) was used for each assay. Measurement of α-HBDH activity was done with Sigma Diagnostics α-HBDB reagent kits formulated according to the recommended 'optimized standard method'.[17] The sample procedure (SSP) was adopted. The absorbance at 340mm was read against a water blank in a UV spectrophotometer. The mean absorbance change per minute (ΔA/min) was used to calculate enzyme activity in units per litre. (One unit HBDH activity is defined as the amount of enzyme that catalyzes the oxidation of 1μmole NADH per minute under the conditions of the assay procedure[17].

Statistical methods

Statistical calculations were carried out manually in a Casio (Model fx-82B) scientific calculator. Mean, standard deviation and 95% confidence interval (CI), where appropriate, were computed. Comparison between groups was performed using the student's *t*-test for unpaired samples. Significance of association between parametric measures was tested by the Pearson's product-moment correlation coefficient.

Results

Among the SCA subjects, the mean age of children in PC 10 ± 4.0 years was comparable to 9 ± 4.0 years for steady state subjects, but in sex distribution there was a higher proportion of boys in the steady state group (2:1) than the PC group (1:1)

In Table 1 the distribution of site of pain among the PC patients is shown. Eight children had generalised pain involving the trunk (abdomen and/or chest and/or lumbosacral region) with or without limb involvement, 4 had pains in two or more long bones involving both upper and lower limbs, 4 in one or more long bones in the upper limbs, 3 in one or more long bones in the lower limbs and 3 had hand-foot syndrome. The haematological indices of the patients and control are summarized in Table 2. There was no difference between SCA patients in PC and those in steady state, in mean venous haematocrit (0.23 ± 0.04 and 0.23 ± 0.05, respectively) or in reticulocyte counts (3.2 ± 2.7 per cent and 4.7 ± 5.1 per cent, respectively). However, 50% of the SCA children in the study had reticulocytopaenia (retics count < 2%). The relationship between α-HBDH level and reticulocyte count is shown in Fig 1. There was no correlation between plasma -α-HBDH level and reticulocyte count ($r = 0.0856$, $t = 0.4565$, $0.6 > P > 0.7$). Plasma enzyme levels in SCA patients and controls is shown in Table 2. The mean α-HBDH level in SCA patients in PC 373.8 ± 113.5 μ/l was significantly higher than 128 ± 19.5 μ/l for controls ($t = 2.8$ on 53 d.f, $P < 0.001$).

Table 1: Site of pain in 21 children with sickle cell anaemia in painful crisis

Site of Pain	Number of Subjects	%
Hand-foot syndrome	2	9.5
Long bones (upper limbs)	4	19.1
Long bones (lower limbs)	3	14.2
Long bones (upper and lower limbs)	4	19.1
Generalised (chest/abdomen ± limb)	8	38.1
Total	21	100

Table 2: α-HBDH levels, haematocrit and reticulocyte counts in SCA patients and controls

Subjects	*n*	α-HBDH μ/l Mean ± SD	Venous Haematocrit Mean ± SD	Reticulocyte (%) Mean ± SD
Control	21	128.3 ± 19.5	0.39 ± 0.05	—
SCA Steady state	34	341.2 ± 103.5	0.23 ± 0.04	3.2 ± 2.7
SCA Painful Crisis	21	373.8 ± 113.5	0.23 ± 0.02	4.7 ± 5.1

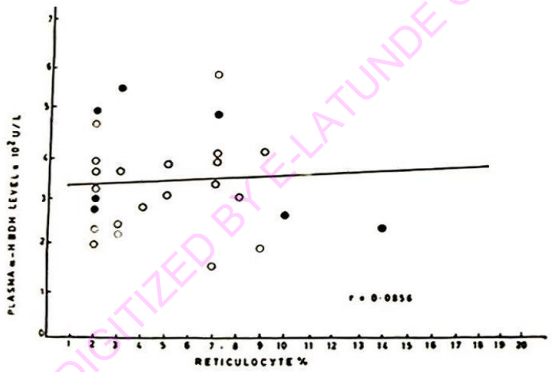

Fig.1: Relationship between plasma α-hydroxybutyrate dehydrogenase level and reticulocyte count in children with sickle cell anaemia. (O, represent steady state, • painful crisis.)

However, the difference between sample mean plasma α-HBDH levels in PC and steady state SCA patients was 32.6μ/l with a 95% confidence interval (CI) from –26 to 91.8μ/l; the *t*-test statistic was 1.095, with 53 degrees of freedom and an associated *P* value of $0.3<P<0.2$.

Thus, α-HBDH level did not discriminate between SCA children in steady state and those in PC.

Discussion

α-HBDH comprises the anodal iso-enzymes 1 and 2 of LDH with high specificity for the reduction of 2-oxobutyrate in the presence of NADH.

Our finding of increased levels of α-HBDH in the plasma of children with SCA during steady state and higher levels during PC is in agreement with earlier studies[12,13]. However, the suggestion that in the absence of cardiac/skeletal muscle or liver disease an elevated plasma level of α-HBDH is due to bone marrow infarction[13,18] is not supported by our data.

We did not find a significant difference between enzyme levels in steady state patients and children in PC, presumably because elevated plasma level of α-HBDH is these children derives from sources other

than infarcted marrow. It is also possible that we were unable to demonstrate a significant difference between steady state patients and those in PC because of the differential clearance rate of LDH isoenzymes. Since LDH_1 is known to be cleared very slowly from circulation[19], the high activity of α-HBDH in the plasma of steady state patients may be attributed to residual LDH_1 from sources other than marrow necrosis.

The sources of plasma LDH iso-enzymes are multiple and include intravascular haemolysis. Mature red blood cells are a rich source of LDH_1 and LDH_2 and in a haemolysate containing 1mg per 100ml of haemoglobin, enzyme activity approximates 2μ/ml of LDH[12]. It can be argued that if PC in our children with SCA was due to malaria infection as had been suggested[6], then the higher levels of α-HBDH observed in their plasma would be a result of intravascular haemolysis associated with malaria infection. The lack of correlation between α-HBDH level and reticulocyte count will not vitiate such argument since reticulocyte count reflects the ability of bone marrow to respond to a given level of anaemia rather than the extent of haemolysis. It should be noted, however, that acute *P. falciparum* infection in some cases may be associated with bone marrow failure.

Megaloblastic haemopoeisis which is also known to produce elevation of LDH_1 and LDH_2[20] was difficult to exclude in our patients since electronic particle counter was not available for accurate mean cell volume (MCV) determination and we did not consider bone marrow examination justified. Nevertheless, because the patients were all on routine folate supplementation and their blood smears did not show hypersegmented neutrophils we consider megaloblastosis unlikely.

Although thrombocytosis is also known to be commonly associated with PC[10] we considered it unlikely that platelet derived LDH iso-enzymes would make significant contributions to α-HBDH levels reported here, since PPP was used, and furthermore, the condition under which our assay was performed is not known to cause significant platelet lysis.

We think that the high plasma α-HBDH is our patients with SCA is most likely due to chronic haemolysis. Since we did not find significant difference in the enzyme levels of PC and steady state patients, we suggest that α-HBDH levels should be used with caution in the diagnosis of PC in Nigerian children.

Acknowledgements

We acknowledge the technical assistance of Mrs B.C. Ogboghodo. We thank Professor A.U. Osagie of the Department of Biochemistry, University of Benin, for his helpful suggestions and Mrs C.N. Ijomah for her secretarial assistance.

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(Accepted 17 October, 1990)