Brief report

Adaptation to anemia in hemoglobin E-β thalassemia

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Hemoglobin E β thalassemia is the commonest form of severe thalassemia in many Asian countries. Its remarkably variable clinical phenotype presents a major challenge to determining its most appropriate management. In particular, it is not clear why some patients with this condition can develop and function well at very low hemoglobin levels. Here, we demonstrate that patients with hemoglobin Eβ thalassemia have a significant decrease in the oxygen affinity of their hemoglobin, that is an increased P_{50} value, in response to anemia. This may in part reflect the lower level of hemoglobin F in this condition compared with other forms of β thalassemia intermedia. The ability to right-shift the oxygen dissociation curve was retained across the spectrum of mild and severe phenotypes, despite the significantly higher levels of hemoglobin F in the former, suggesting that efforts directed at producing a modest increase in the level of hemoglobin F in symptomatic patients with this disease should be of therapeutic value. (*Blood*. 2010;116(24): 5368-5370)

Introduction

Globally, approximately half of the clinically important forms of β thalassemia result from the compound heterozygous inheritance of hemoglobin (Hb)E and β thalassemia, HbE-β thalassemia, a condition that occurs commonly in Asia and constitutes an increasing proportion of patients with thalassemia in immigrant populations in the United States and elsewhere.[1,2] Because of its phenotypic instability in early life and its remarkable clinical variability, ranging from a transfusion-dependent disorder similar to β thalassemia major to a condition compatible with normal growth and development without transfusion, it presents considerable management problems.[3-5] Although some of the genetic modifiers that are responsible for its phenotypic variability have been identified,[5-7] and its early phenotypic instability has been ascribed to age-related variation in erythropoietin response to anemia,[8] one of the most puzzling features of this condition is why so many mildly affected patients can function and develop so well with extremely low hemoglobin levels.[3,5] Here, we present evidence that, at least in part, this may reflect a more effective ability to respond to anemia than occurs in other intermediate forms of β thalassemia.

Methods

Patients

Fifty-six patients aged 2-46 years with HbE-β thalassemia under long-term observation at the National Thalassemia Center, Kurunegala, Sri Lanka, and who had never received transfusion or not been transfused for at least 3 months, were studied. Methods for their clinical assessment and classification into different severity groups have been reported previously.[3] In short, the mild group comprised 36 patients who were fully active and had grown and developed satisfactorily without transfusion, or those with a similar phenotype observed for at least 6 years after they had been taken off transfusion. The severe group of 20 patients had not been able to function or develop adequately without transfusion. For comparison, 30 normal Sri Lankan or European controls were studied together with a small group of patients with different forms of β thalassemia intermedia or sickle-cell disorders who were attending the same clinic. Those classified as having β thalassemia intermedia were homozygous for severe β thalassemia mutations but had been maintained without transfusion, almost certainly because of a so far unexplained increased ability to produce HbF. Those with δβ thalassemia were heterozygous for either HbE or β thalassemia and a novel deletion involving the *HBB* and *HBD* loci that will be described in detail elsewhere. In addition, for reference purposes the oxygen affinity of 6 cord blood samples was determined.

Methods

A 1.5-mL heparinised blood sample was obtained for duplicate measurement of blood gases, pH, hemoglobin and P_{50} using a Rapidpoint 405 analyser with an integral co-oximeter (Bayer). The remainder was deproteinised with 0.6M perchloric acid and neutralised with 2.5M potassium carbonate; supernants were stored at −20°C and shipped to Oxford on dry ice for measurement of 2,3-biphosphoglycerate (2,3BPG) levels (Roche Diagnostics). A further 2.0-mL blood sample was collected for hematologic analysis (Coulter Electronics) and identification of hemoglobin variants by high-performance liquid chromatography (HPLC; BioRad).

The plasma was then separated from the cells and both stored at −20° and shipped to Oxford on dry ice for measurement of plasma erythropoietin levels by enzyme-linked immunosorbent assay (R&D Systems) and for DNA analysis of the *HBB* genes.[9]

Statistical analysis

Univariate and multiple regression analyses were performed using STATA 11 software or SPSS 16 (SPSS Inc). Because some variables were not

Submitted June 8, 2010; accepted August 26, 2010. Prepublished online as *Blood* First Edition paper, September 10, 2010; DOI 10.1182/blood-2010-06-289488.

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Table 1. Basic data for patients with HbE β thalassemia, controls and related diseases

	n	Hb (g/dl)	$P_{50\ (mmHg)}$	2,3-BPG (μmol/gHb)	Erythropoietin (mIU/ml)	HbF (%)	HbF (g/dl)
HbE β thalassemia							
Mild	36	5.6 (3.7-8.8)	28.2 (22.6-31.2)	22.7 (9.39-44.4)	236.5 (39.4-1675.8)	28.6 (6.2-51.4)	1.61 (0.38-3.69)
Severe	20	4.9 (3.2-7.4)	27.8 (25.1-30.2)	27.2 (7.9-48.2)	462.2 (53.8-7724.4)	26.9 (6.2-46.5)	1.31 (0.43-2.74)
Total	56	5.4 (3.2-8.8)	27.9 (22.6-31.2)	24.0 (7.9-48.2)	256.0 (39.4-7724.4)	28.1 (6.2-51.4)	1.52 (0.38-3.69)
Hb E δβ thalassemia	1	10.3	24.7	21.9	25.5	48.7	5.02
β thalassemia intermedia	2	4.0 5.4	21.8 22.0	19.9 27.7	10366.1 19158.7	94.6 78.5	3.78 4.23
β/δβ thalassemia	3	8.0 9.96.9	21.9 19.5 22.5	17.0 14.7 11.9	356.8 53.6 571.4	80.0 86.4 61.8	6.4 8.55 4.26
Hb S β thalassemia	1	7.5	26.9	33.1	159.8	18.5	1.39
Sickle cell anemia	2	9.5 8.1	30.3 30.7	44.2 28.9	58.6 39.9	13.6 11.3	1.29 0.91
Hb SE disease	1	9.5	26.9	15.0	54.1	21.2	2.0
Hb SD disease	1	9.1	30.6	20.8	59.0	9.7	0.88
Healthy controls	30	14.0 (10.6-16.3)	25.6 (24.1-27.2)	13.2 (8.8-19.6)	17.5 (1.03-25.7)	0.70 (0.5-5)	0.10 (0.05-0.81)

Values are shown for individuals where 3 or fewer were tested; otherwise median and range are reported.

normally distributed median values are reported and the difference assessed using the Mann-Whitney U test.

Ethical approval

Approval for the research program on HbE β thalassemia was obtained from the Ethical Committee of the College of Pediatricians, Colombo, Sri Lanka, and the Oxford Tropical Research Ethical Committee.

Results and discussion

The data are summarized in Table 1. The 56 patients with HbE-β thalassemia were heterozygous for both HbE and one of the severe β thalassemia alleles that are common in Sri Lanka, notably IVS1-5 (G-C), IVS1-1 (G-A), and CD41/42 (-TCTT).[9]

There were no significant differences between the partial pressure at which hemoglobin is half saturated with oxygen (P_{50}) values or any other parameters between the European and Sri Lankan controls, compared with whom the patients with HbE-β thalassemia showed a significant increase in their P_{50} values ($P = .001$). Univariate analysis demonstrated a highly significant reduction in P_{50} values associated with increased levels of HbF, whether expressed as percentage ($P = .001$) or g/dL ($P = .001$). Conversely, increasing 2,3-BPG levels were strongly associated with increased levels of P_{50} ($P = .006$). The correlation between the P_{50} and hemoglobin level was less significant ($P = .065$) and there was no correlation between the P_{50} value and the erythropoietin level. Multiple regression analysis comparing P_{50} values with hemoglobin HbF and 2,3-BPG confirmed these relationships and that both HbF and 2,3-BPG levels are both strongly associated with P_{50}, pulling in opposite directions, while the hemoglobin level is less strongly associated with the P_{50}.

The low P_{50} levels in the patients with β thalassemia intermedia, and the increased values in the different sickle-cell variants studied here, are similar to those previously reported.[6,10,11] The P_{50} levels in the patients with δβ/β thalassemia, like those with β thalassemia intermedia, were also extremely low and similar to the values obtained from 6 umbilical cord bloods (P_{50} 18.6-21.1, mean 19.6).

Earlier studies showed that HbE in intact red cells or dilute solution has a normal oxygen affinity and interacts normally with 2,3-BPG.[12-14] In a single case of HbE β thalassemia, the P_{50} was remarkably elevated although the level of HbF in this patient, approximately 2%, was unusually low.[14] The present study shows that, overall, patients with HbE β thalassemia are able to adapt to anemia by reducing the oxygen affinity of their red cells. Their degree of adaptation clearly reflects the very strong effect of high levels of 2,3-BPG in reducing the oxygen affinity counterbalanced by the equally strong effects of the high affinity of HbF pulling in the opposite direction. It seems likely therefore that at least one reason why patients with HbE thalassemia are able to adapt better to severe anemia than those with other forms of β thalassemia intermedia reflects the relatively low HbF levels in this condition. Considering these complex interactions, the wide age spread in the population studied here, and our previous observations about the changes in erythropoietin response to anemia at different ages,[8] it is not surprising that, although there was a reasonable correlation between the level of erythropoietin and hemoglobin, there was no correlation with the P_{50} value.

The findings in the mild and severe phenotypes, are summarized in Table 1. Although the hemoglobin level was significantly higher in the mild group ($P = .001$), this only reflects a difference of approximately 1g/dL, a surprising finding that has been consistent throughout all our studies of this disease in Sri Lanka.[3,5,15] This lower mean hemoglobin level in the mildly affected cases compared with those in other populations may, at least in part, reflect the absence of the interaction of mild β thalassemia alleles with HbE in Sri Lanka. Although the P_{50} tended to be higher in the mild cases, this difference was not statistically significant. However, the 2,3 BPG levels and erythropoietin levels were higher in the severe group ($P = .022$ and .037, respectively)

As we have previously observed,[15] the HbF levels in the mild group of patients were significantly higher than in the severe group ($P = .025$). In absolute terms, that is g/dL, this is reflected in the higher hemoglobin levels in the mild group. However, the P_{50} values in both mild and severe groups are significantly higher than in the normal control populations and very much higher than those of the patients with the other forms of β or δβ thalassemia intermedia. It appears therefore that a modest increase in HbF, whether it is due to the action of genetic modifiers or might be achieved by current therapeutic approaches to elevate HbF levels is of genuine clinical value in HbE β thalassemia. However, and particularly in view of current therapeutic efforts to raise the output of HbF in different hemoglobinopathies, further studies are required to define the threshold values of HbF at which the functional effects of a decreased P_{50} counterbalance the potential improvements in effective erythropoiesis and a higher Hb level that result from increased γ globin chain production.

These observations raise further important questions about the phenotypic diversity of HbE β thalassemia. In particular, while the known genetic modifiers such as HbF and α thalassemia[6] clearly

play a role in determining the phenotype, and while the ability to right-shift the oxygen dissociation curve may contribute to more effective adaptation to low hemoglobins right across the spectrum of phenotypes, given the small difference in hemoglobin levels between the different phenotypes in this group of patients with uniformly severe β thalassemia mutations interacting with HbE, other factors, possibly related to adaptation to anemia, must be involved.

A better understanding of these adaptive mechanisms is extremely important because, as seems likely, many children with this condition are being placed on transfusion based on their hemoglobin level, with the potential waste of valuable resources, particularly in poorer countries in which HbE-β thalassemia is so common.

Acknowledgments

We thank Jeanne Packer and Liz Rose for their help in preparing this manuscript.

This work was supported by grants from the United Kingdom Wellcome Trust and the US March of Dimes. T.P. is funded by the NIHR Oxford Biomedical Research Center.

Authorship

Contribution: A.A. and C.F. carried out the laboratory studies; T.P. and S.A. carried out the statistical analyses; A.P, V.T., M.A., and N.O. collected and analyzed the clinical data on the families, and D.W. designed and directed the project and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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