Adaptation to anemia in hemoglobin E-β thalassemia

Angela Allen1,2, Christopher Fisher1, Anuja Premawardhena3, Timothy Peto1,
Stephen Allen2, Mahinda Arambepola4, Vivekanandan Thayalsutha5, Nancy Olivieri5,
and David Weatherall1

1 Weatherall Institute of Molecular Medicine, University of Oxford, John
Radcliffe Hospital, Headington, Oxford, OX3 9DS, United Kingdom.
2 School of Medicine, University of Wales, Swansea, SA2 8PP, United Kingdom.
3 University of Kelaniya, Colombo, Sri Lanka.
4 National Thalassaemia Centre, District Hospital, Kurunegala, Sri Lanka.
5 Hemoglobinopathy Research, University Health Network, 200 Elizabeth Street,
Toronto, ON, Canada, M5G 2C4.

Correspondence: Professor Sir David Weatherall, Weatherall Institute of
Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington,
Oxford. OX3 9DS e.mail: < liz.rose@imm.ox.ac.uk >
Abstract

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 HbEβ thalassemia have a significant decrease in the oxygen affinity of their hemoglobin, that is an increased P50 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.

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 USA 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,\textsuperscript{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.\textsuperscript{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 $\beta$ thalassemia.

**Patients and Methods**

*Patients.* Fifty-six patients aged 2 – 46 years with HbE-$\beta$ thalassemia under long-term observation at the National Thalassemia Centre, Kurunegala, Sri Lanka, and who had never received transfusion or not been transfused for at least three months, were studied. Methods for their clinical assessment and classification into different severity groups have been reported previously.\textsuperscript{3} In short, the ‘mild’ group was comprised of 36 patients who were fully active and had grown and developed satisfactorily without transfusion, or those with a similar phenotype observed for at least six 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 $\beta$ thalassemia intermedia or sickle-cell disorders who were attending the same clinic. Those classified as having $\beta$ thalassemia intermedia were homozygous for severe $\beta$ thalassemia mutations but had been maintained without transfusion, almost certainly because of a so-far unexplained increased ability to produce HbF. Those with $\delta\beta$ thalassemia were heterozygous for either HbE or $\beta$ 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 six cord blood samples was determined.

*Methods.* A 1.5ml 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, Newbury, UK). 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, Penzberg, Germany). A further 2.0ml blood sample was collected for hematologic analysis (Coulter Electronics, UK) and identification of hemoglobin variants by high performance liquid chromatography (HPLC) (BioRad, India).

The plasma was then separated from the cells and both stored at -20°C and shipped to Oxford on dry-ice for measurement of plasma erythropoietin levels by ELISA (R and D Systems, Abingdon, UK) 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., Chicago, Il). Since some variables were not 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 P50 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 ($p = 0.001$).

Univariate analysis demonstrated a highly significant reduction in $P_{50}$ values associated with increased levels of HbF, whether expressed as percentage ($p = 0.001$) or g/dl ($p = 0.001$). Conversely, increasing 2,3-BPG levels were strongly associated with increased levels of $P_{50}$ ($p = 0.006$). The correlation between the $P_{50}$ and hemoglobin level was less significant ($p = 0.065$) 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 six 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, about 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 (\(p=0.001\)), this only reflects a difference of about 1 g/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 \(\beta\) 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 (\(p=0.022\) and 0.037 respectively).

As we have previously observed\(^{15}\), the HbF levels in the mild group of patients were significantly higher than in the severe group (\(p=0.025\)). 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 \(\beta\) or \(\delta\beta\) 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 \(\beta\) 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 \(\gamma\) 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.

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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.V, and N.O. collected and analysed 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 financial or other competing interests.

Correspondence: Professor Sir David Weatherall, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford. OX3 9DS e.mail: < liz.rose@imm.ox.ac.uk >
References


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<th>P50 (mmHg)</th>
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<th>Erythropoietin (mIU/ml)</th>
<th>HbF (%)</th>
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<td>Mild</td>
<td>36</td>
<td>5.6</td>
<td>28.2</td>
<td>22.7</td>
<td>236.5</td>
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<td>[39.4-1675.8]</td>
<td>[6.2-51.4]</td>
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<td>Severe</td>
<td>20</td>
<td>4.9</td>
<td>27.8</td>
<td>27.2</td>
<td>462.2</td>
<td>26.9</td>
<td>1.31</td>
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<td>[25.1–30.2]</td>
<td>[7.9-48.2]</td>
<td>[53.8-7724.4]</td>
<td>[6.2-46.5]</td>
<td>[0.43-2.74]</td>
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<td>Total</td>
<td>56</td>
<td>5.4</td>
<td>27.9</td>
<td>24.0</td>
<td>256.0</td>
<td>28.1</td>
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<td>[22.6-31.2]</td>
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<td>15.0</td>
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<tr>
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<td>[0.5-5]</td>
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**Table 1. Basic data for patients with Hb E β thalassemia, controls and related diseases**
Values are shown for individuals where 3 or fewer were tested; otherwise median and [range] are reported.
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