On the pathophysiology of severe falciparum malaria with special reference to red cell deformability
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Red cell deformability, splenic function and anaemia in thalassaemia

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Red cell deformability (RCD) was measured in 38 patients with α-thalassaemia and 48 patients with β-thalassaemia, of whom 13 had undergone splenectomy. All splenectomized patients, but none of those with intact spleens, had very rigid erythrocytes with an elongation index <0.45 at a high shear stress of 30 Pa suggesting a splenic recognition threshold for removal of rigid red cells. At this shear stress RCD correlated strongly with the degree of anaemia in both the splenectomized \((r=0.81, p<0.001)\) and non-splenectomized β-thalassaemic patients (all patients \(r=0.81, p<0.001\); homozygous β-thalassaemic patients \(r=0.51, p=0.01\)). These data suggest that reduced RCD is a major determinant of anaemia in thalassaemia.
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Introduction

Thalassaemia is associated with dyserythropoiesis and anaemia which ranges in severity from very mild to lethal\(^1\). Reduced red cell deformability (RCD) is considered an important determinant of the erythrocyte life span within the circulation in both physiological red cell ageing and in disease\(^2\). Although reduced RCD has been well documented in thalassaemia\(^3\), there have been no studies correlating quantitatively the reduction in red cell deformability and the severity of anaemia in thalassaemia. We have measured RCD at a range of shear stresses, to assess the importance of reduced RCD in thalassaemia and the role of the spleen in red blood cell processing.

Materials and methods

The study was performed in Mahidol University, Bangkok, Thailand. Different groups of well characterised thalassaemic patients attending the haematology department of Siriraj Hospital, Bangkok, Thailand were studied provided that the patients gave informed consent to providing a blood sample (1ml). The diagnosis of thalassaemia was based on globin gene analysis and haemoglobin electrophoresis on an automated HPLC system (VARIANT®, Bio-Rad)\(^4\). None of the patients had received a blood transfusion in the previous three months. Seventeen healthy age matched Thai subjects served as a control group. A routine haematology blood sample was used for studies of RCD which was measured immediately after venepuncture with a Laser-assisted Optical Rotational Cell Analyser (LORCA, Mechatronics, The Netherlands)\(^5\). Red cell deformability was measured at two shear stresses; 30 Pa corresponding approximately to the stresses anticipated in the spleen and 1.7 Pa, corresponding approximately to the stresses on the arterial side of the circulation. The unit of deformability is the elongation index (E.I.) defined as the ratio between the difference between the two axes of the ellipsoid diffraction pattern and the sum of these two axes. The diffraction pattern is directly proportional to the mean ellipticity of the erythrocytes under the shear stress applied. Haemoglobin concentrations, mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were measured by an automatic blood cell counter (Cymex 1500).

Results

The following groups were studied; heterozygous β-thalassaemia (n=10), homozygous β-thalassaemia intermedia (n=38), of whom 13 had undergone splenectomy, HbH disease (n=14), HbH/Constant Spring (CS) (n=10), homozygous HbCS (n=6), and heterozygous
α1-thalassaemia (trait) (n=6). The mean (SD) age of the patients was 39 (10) years and did not differ significantly between the different groups.

β-thalassaemia

In all patients with β-thalassaemia there was a strong correlation between the mean RCD at a high shear stress of 30 Pa and the blood haemoglobin concentrations (splenectomized homozygous β-thalassaemia: \( r=0.81 \), adjusted \( r^2 = 0.62 \), \( p=0.001 \); non-splenectomized homozygous β-thalassaemia: \( r=0.51 \), adjusted \( r^2 = 0.22 \), \( p=0.01 \); heterozygous β-thalassaemia: \( r= 0.83 \), adjusted \( r^2 = 0.64 \), \( p<0.001 \); all patients with intact spleen: \( r=0.81 \), adjusted \( r^2 = 0.65 \), \( p<0.001 \), fig. 1). All splenectomized patients, but none of those with intact spleens had an RCD below E.I.=0.45 at a shear stress of 30 Pa; mean E.I. (SD): 0.358 (0.037) compared to 0.522 (0.025) respectively. In contrast no difference in the RCD of splenectomized versus non-splenectomized patients was found at a low shear stress of 1.7 Pa, and the correlation between the RCD and the haemoglobin concentrations weakened considerably (\( r=0.49 \), \( p=0.03 \), fig. 2). There was also a significant correlation between the MCHC and the RCD at 30 Pa (\( r=0.63 \), \( p<0.001 \)), but not between MCV and RCD at this level of shear.

α-thalassaemia

RCD in α-thalassaemia was slightly reduced compared to healthy controls. At a shear stress of 30.0 Pa, the E.I. (SD) for the different groups was: HbH 0.562 (0.035), HbH/CS 0.571 (0.030), Hb CS/CS 0.587 (0.023) and heterozygous α1-thalassaemia (trait) 0.607 (0.008) versus 0.610 (0.010) in the control group. In contrast with the patients with β-thalassaemia, there was only a weak correlation between RCD at a shear stress of 30 Pa and haemoglobin concentrations in the group with α-thalassaemia (\( r=0.48 \), adjusted \( r^2 = 0.23 \), \( p<0.002 \), fig. 3). There was no correlation at lower levels of shear. As in β-thalassaemia there was a significant correlation between the MCHC (but not the MCV) and the RCD at 30 Pa (\( r=0.55 \), \( p<0.001 \)).

Discussion

The thalassaemias form a heterogeneous group of genetic abnormalities characterised by deficient synthesis of one or more of polypeptide chains of the normal haemoglobin, resulting in excess α-chain to β-chain formation in β-thalassaemia, and the opposite in the α-thalassaemias. Precipitation of the unstable globins and their attachment to the membrane skeleton cause oxidation of the skeletal proteins and rigidification of the red cell membrane. In haemoglobin Constant Spring, a nondeletional α-thalassaemia mutation, increased membrane rigidity is related to attachment of both oxidised β-globin chains and αCS-globin chains to the membrane.
Figure 1. Correlation between red cell deformability (RCD) at a high level of shear stress (30 Pa.) and the severity of anaemia in splenectomized and non-splenectomized patients with β-thalassaemia.

- • = splenectomized patients with homozygous β-thalassaemia (n=13).
- ○ = non-splenectomized patients with homozygous β-thalassaemia (n=25).
- □ = patients with heterozygous β-thalassaemia (n=10).
- ▐ = healthy controls (n=17)

E.I. = elongation index.

Figure 2. Correlation between red cell deformability (RCD) at a low level of shear stress (1.7 Pa.) and the severity of anaemia in splenectomized and non-splenectomized patients with β-thalassaemia.

- • = splenectomized patients with homozygous β-thalassaemia (n=13).
- ○ = non-splenectomized patients with homozygous β-thalassaemia (n=25).
- □ = patients with heterozygous β-thalassaemia (n=10).
- ▐ = healthy controls (n=17)

E.I. = elongation index.

Figure 3. Correlation between red cell deformability (RCD) at a high level of shear stress (30 Pa.) and the severity of anaemia in patients with α-thalassaemia.

- • = HbH disease
- △ = HbH/ Constant Spring (CS)
- □ = homozygous HbCS
- △ = heterozygous α1-thalassaemia (trait)
- ▐ = healthy controls (n=17)

E.I. = elongation index.
Anaemia is the central feature of the thalassaemias and results from dyserythropoiesis with intramedullary haemolysis and increased (splenic) clearance of red cells in the peripheral blood. This shortened red cell survival has been attributed to the reduction in RCD. We found a direct correlation between the reduction in RCD at high levels of shear and the severity of anaemia in β-thalassaemic patients suggesting a causal relationship. High shear stresses are found in splenic sinusoids, where erythrocytes have to squeeze through narrow slits with a width of 0.5-2 μm in order to enter the sinusoids of the spleen.

The correlation between RCD and anaemia was weaker in the α-thalassaemic group, possibly because the red cells were less rigid than in β-thalassaemia. Also, erythropoiesis is more effective in this group. Alternatively other mechanisms are responsible for the increased clearance of erythrocytes in this group.

All the splenectomized β-thalassaemic patients had RCD values below E.I.=0.45 at the high shear stress of 30 Pa., whereas none of those with intact spleens did. This difference was not present at lower levels of shear. If the spleen is removed very abnormal red cells, containing inclusion bodies, continue to circulate. This suggests that the splenic removal threshold for rigid erythrocytes in β-thalassaemia corresponds with an E.I. of 0.45 at a shear stress of 30 Pa. In the splenectomized patients RCD remained correlated with the degree of anaemia, indicating that RCD still remains an important determinant of red cell survival in the absence of splenic clearance. This study suggests that reduced RCD is an important contributor to anaemia in thalassaemia.

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References
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