Spectroscopic study of red blood cells by confocal microscopy in patients with thalassemia: Preliminary results.

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Red blood cell disorders can result in severe complications if they are not diagnosed in time, which is especially sensitive and critical in children. In thalassemia, the synthesis of hemoglobin is quantitatively altered being one of the types of globin chain produced sparingly or not produced at all causing the excess and aggregation of the other type of globin chain. Patients affected by this disorder suffer from mild symptoms such as joint pain, fever and infections, to more severe symptoms such as eye diseases, growth restriction, and, in the most severe cases, bone marrow deformities and organ elongations. Some of them are irreversible, so it is very important to give an accurate and fast diagnosis at an early age. Since traditional microscopy usually cannot give a final diagnosis for this disease, in this work, we explored the possibilities of confocal microscopy as a diagnostic tool in patients with thalassemia. For this purpose, blood samples from patients with alpha-thalassemia were evaluated. In addition, a patient with iron deficiency anaemia, a disease in which oxygen transport is impaired due to malabsorption or loss of iron, and a patient without blood diseases (control) were included. Spectral imaging was performed using a TCS SP8 confocal laser-scanning microscope (Leica Microsystems GmbH, Mannheim, Germany) equipped with a detection unit that allows spectral discrimination using 63X (NA 1.4, oil) Plan-Apochromatic objective and hybrid detectors. Blood samples were excited with a laser diode at 405 nm and the fluorescence emission spectra were collected from 425 to 780 nm with 20 nm bandwidth (step size =7 nm). The results showed two fluorescence emission peaks at 628 nm and 649 nm for the patients with thalassemia and ferropenic anaemia, whether the last peak at 649 nm did not appear in the control patient. The ratio between the autofluorescence peaks allowed a preliminary discrimination among healthy and diseased patients, although much larger studies are required to establish cut-off points. Future work will consist on expanding the set of samples of both alpha-thalassemia and iron deficiency anaemia, and exploring them in terms of reflectance.