Prior to the introduction, it is important to understand the background and context of the study. Sickle cell disease (SCD) is a genetic disorder that affects the production of hemoglobin, a protein in red blood cells (RBCs) that carries oxygen throughout the body. SCD arises from an inherited mutation in the beta globin gene. The most common form of SCD is homozygous sickle cell disease (HbSS), or sickle cell anemia. The population of people with HbSS is expanding worldwide, as are novel therapies for its treatment. It is predicted that the number of newborns with SCD who are born per annum will increase from approximately 350,000 in 2010 to 445,000 in 2050 [1].

Priapism is a serious, but episodic, complication of sickle cell disease (SCD). We had previously reported that subjects with SCD had variable red blood cell (RBC) adhesion to the immobilized sub-endothelial protein laminin (LN). We examined adhesion to LN in a microfluidic device, of RBCs from men with homozygous sickle cell anemia. Adhesion under hypoxic, but not ambient, conditions was greater in men with a history of priapism, with median adhesion of 529 RBCs per 32 mm²/unit area (range 5–5248) rising to 3268 RBCs per 32 mm²/unit area (range 49–18,368, P = 0.004), under ambient and hypoxic conditions, respectively (n = 14). This was not seen in RBCs from men without a history of priapism (median 402 (range 14–785) and 122 (range 31–4112) RBCs per 32 mm²/unit area, ambient and hypoxic conditions, respectively (P = N.S., N = 12)). We also observed an association between hypoxia-enhanced RBC adhesion in vitro and a history of hemoglobin desaturation in vivo independent of priapism. Prolonged Hb desaturation may increase sickle polymer formation and RBC damage, resulting in enhanced RBC adhesion, hemolysis, and endothelial dysfunction. The identification of distinct RBC phenotypes could prompt clinical evaluation for suitability for novel or under-used therapies, like oxygen.
microfluidic platform (SCD Biochip) to measure RBC adhesion under physiologic flow conditions [12]. For hypoxia experiments, we integrated this platform with a micro-gas exchanger that exposed the blood to low oxygen conditions prior to being flown across the SCD biochip, resulting in desaturation of Hb in the sample (to an SpO2 of 83%) [9].

2. Materials & Methods

Surplus blood draw samples, collected in ethylenediaminetetraacetic acid (EDTA), were obtained from people with SCD during non-crisis clinic visits at the Adult Sickle Cell Disease Clinic at University Hospitals Seidman Cancer Center/CWRU in Cleveland, OH. All de-identified samples were processed within 24 h. Clinical information, including treatment, medical history, and laboratory tests, was obtained from the electronic medical records system. We recorded a history of priapism, at any time, and any evaluation for hemoglobin desaturation sufficient to warrant prescription of oxygen within 5 years, e.g. 6-minute walk distance test, sleep study, or overnight oximetry. Laboratory tests, obtained at non-crisis clinic visits, included a complete blood count (CBC), comprising white blood cell count (WBC, 10^9/L), platelet count (10^9/L), and absolute neutrophil count (ANC, 10^9/L), as well as reticulocyte count (10^9/L), lactate dehydrogenase levels (LDH, U/L), ferritin levels, a renal panel, liver function tests, hemoglobin S (HbS) %, hemoglobin F (HbF) %, and total hemoglobin (g/dL). Pain level data, on a numerical scale from 0 (no pain) to 10 (highest pain), were routinely collected at each clinic visit. Concomitant treatments such as hydroxyurea or transfusions were recorded. All studies were performed with institutional review board approval and written informed consent from subjects.

The SCD Biochip was designed to recapitulate volume and flow of post-capillary venules. It was functionalized with LN and blocked with 2% BSA to prevent non-specific adhesion [9–12]. The microchannels were connected with inlet tubing which was non-permeable (ambient conditions), or gas permeable within non-permeable tubing, which contained 5% CO2 and 95% N2. As previously reported, 15 μL of undiluted EDTA-anticoagulated blood sample was injected into the channels through the inlet tubing at an approximate shear stress of 0.1 Pa, and non-adherent cells were rinsed out by flowing a wash buffer solution. Phase-contrast images of the microchannels with adherent RBCs were recorded with an inverted microscope, and adherent RBCs were manually quantified with Adobe Photoshop software (San Jose, CA).
CA) in a 32 mm² window [9–12].

A test of normality was performed on relevant variables. Non-normally and normally distributed variables were described with medians and ranges. Appropriate two-sample tests were used to compare groups, t-tests for normally distributed values and Mann-Whitney for non-normally distributed variables. Appropriate paired t-tests were used to compare the change from normoxic and hypoxic states. This was not seen in men without a history of priapism (402 (range 14–785) and 122 (31–4112, N=12) RBCs/32 mm²/unit area, under ambient and hypoxic conditions, respectively (P=N.S., not shown). Of note, there was no significant difference in baseline adhesion between these groups under ambient conditions (P=N.S., not shown).

Although there was a trend towards Hb desaturation in vivo in men with a history of priapism (Table 1), its presence, analyzed primarily, was highly associated with hypoxia enhanced RBC adhesion in vitro, regardless of priapism history. Men with a clinical history of Hb desaturation in vivo showed hypoxia-enhanced adhesion to LN, rising from a median 440 (range 16–5248) to a median 4940 (range 16–5248) RBCs/32 mm²/unit area, under ambient and hypoxic conditions, respectively (P=0.002, Fig. 1). Of note, there was no significant difference in baseline adhesion between these groups under ambient conditions (P=N.S., not shown).

Men with a history of priapism had evidence for chronic inflammation (elevated WBC and neutrophil counts and elevated ferritin levels) and hemolysis (elevated LDH levels, Table 1). In separate K-means clustering analyses, men with HbSS and a history of priapism were more likely to have a history of priapism than were men with a less hemolytic phenotype (Fig. 1 supplement).

Men with a history of priapism showed significantly hypoxia-enhanced adhesion to LN, rising from a median of 529 RBCs/32 mm²/unit area (range 5–5248) under ambient conditions to 3268 RBCs/32 mm²/unit area under hypoxic conditions (range 49–18,368, P=0.004, N=14). This was not seen in men without a history of priapism (402 (range 14–785) and 122 (31–4112, N=12) RBCs/32 mm²/unit area, under ambient and hypoxic conditions, respectively (P=N.S., Fig. 1). Of note, there was no significant difference in baseline adhesion between these groups under ambient conditions (P=N.S., not shown).

3. Results

Paired RBC samples from men with a history of priapism showed hypoxia-enhanced adhesion to LN, rising from a median of 529 RBCs/32 mm²/unit area (range 5–5248) under ambient conditions to 3268 RBCs/32 mm²/unit area under hypoxic conditions (range 49–18,368, P=0.004, N=14). This was not seen in men without a history of priapism (402 (range 14–785) and 122 (31–4112, N=12) RBCs/32 mm²/unit area, under ambient and hypoxic conditions, respectively (P=N.S., not shown).

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References


