EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Decreased Hepcidin Levels Are Associated with Low Steady-state Hemoglobin in Children With Sickle Cell Disease in Tanzania

Nathaniel Lee [a], Julie Makani [b,c], Furahini Tluway [b], Abel Makubi [c], Andrew E. Armitage [d], Sant-Rayn Pasricha [d], Hal Drakesmith [d], Andrew M. Prentice [e], Sharon E. Cox [a,e,*]

[a] School of Tropical Medicine & Global Health, Nagasaki University, Nagasaki, Japan
[b] Sickle Cell Programme, Muhimbili University of Health & Allied Sciences, Dar-es-Salaam, Tanzania
[c] Department of Haematology & Blood Transfusion, Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, Tanzania
[d] MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, UK
[e] London School of Hygiene and Tropical Medicine, London, UK

ARTICLE INFO

Article history:
Received 25 February 2018
Received in revised form 17 July 2018
Accepted 17 July 2018
Available online xxxx

Keywords:
Sickle cell disease
Nutrition
Iron metabolism
Hepcidin
Sickle cell anemia
Sub-Saharan Africa

ABSTRACT

Background: The contribution of hepcidin as a regulator of iron metabolism & erythropoiesis on the severity of anemia in sickle cell disease (SCD) remains poorly characterized, especially in Sub-Saharan African populations. The aims of the study were to determine if hepcidin is associated with severity of steady-state anemia in SCD and to investigate factors associated with hepcidin and anemia in SCD.
Methods: Archived samples from 199 Tanzanian children, 56% boys aged 3–18 with laboratory-confirmed SCD were analysed based on recorded averaged steady-state hemoglobin (ASSH) quartiles (lowest vs. highest). Univariable and multivariable logistic regression was used to assess associations with ASSH quartiles.
Findings: In univariable analysis, hepcidin <5·5 ng/mL was associated with increased odds of being in the lowest ASSH quartile (OR 2·20; 95%CI 1·2–3·93) but which was limited to girls (OR 4·85, 95%CI 1·79–13·09, $p = .046$ for interaction). In multivariable analyses including either reticulocyte percentage or erythropoietin, lower hepcidin remained significantly associated with lowest ASSH quartile, although the hepcidin-sex interaction no longer reached statistical significance. No associations with ASSH quartile were observed for markers of inflammation, hemolysis or potential iron markers except for microcytosis, associated with higher ASSH, but which was confounded by reticulocyte percentage and alpha-thalassaemia status.
Interpretation: Hepcidin is lower in more severely anaemic children with SCD independent of inflammation or markers of erythropoiesis.
Funding: Funding sources include The Wellcome Trust (080025, 095009, 094780 & 070114), MRC-UK (MC-A760-5QX00), NIHR Oxford Biomedical Research Centre, and the Bill and Melinda Gates Foundation ("Hepcidin and Iron in Global Health", OPP1055865).

Research in Context

Sickle cell disease (SCD) is one of the most common inherited disorders globally, affects hemoglobin production, and is a major cause of child mortality and poor health, most prominently in Sub-Saharan Africa. Anemia is a defining feature of SCD, but the relative causes of the degree of severity are incompletely understood. Hepcidin downregulates iron absorption and supply to tissues in inflammation and infection and levels decrease when iron is limited or red cell production is increased or under low oxygen conditions. In this study, we show that low hepcidin was associated with severe anemia in children with SCD, independent of markers of inflammation or erythropoietic drive and that this effect appeared to be limited to girls.

1. Introduction

Sickle cell disease (SCD) is one of the most common inherited diseases with the majority of the burden occurring in Sub-Saharan Africa and India, settings where iron deficiency is also common. [1] SCD has a range of clinical manifestations, including anemia from increased hemolysis. [2] SCD is also associated with an increased risk of mortality,

* Corresponding author at: London School of Hygiene & Tropical Medicine, London, UK.
E-mail address: sharoncox@lshtm.ac.uk (S.E. Cox).

https://doi.org/10.1016/j.ebiom.2018.07.024

contributing significantly to under-five mortality in Sub-Saharan Africa. [3]

Low iron status has not been considered a significant contributor to the degree of severity of anemia in SCD as it is assumed that iron absorption would increase to meet the increased erythropoietic need. Furthermore, as children with SCD may receive repeated blood transfusions, iron overload is more of a concern. [4] An increased understanding of the regulation of iron metabolism, and in particular the role of hepcidin a 25-amino-acid peptide hormone synthesized by hepatocytes as a key negative-feedback regulator of iron status, suggests that sickle-related processes of increased erythropoiesis and inflammation may have conflicting effects on hepcidin and hence iron status. [5] Hepcidin blocks iron absorption across the gut and iron efflux from the reticuloendothelial system. Hepcidin expression is increased by inflammation and results in a hypoferremic state, which is an important anti-infective response. [6] Hepcidin expression is decreased by erythropoiesis and hypoxia, thereby increasing iron absorption and release of body iron to meet increased erythron demand. [6]

The contribution of iron deficiency to anemia in patients with SCD living in areas where nutritional iron deficiency anemia is common is unknown. The measurement of iron status is complicated by chronic inflammation and increased erythropoiesis that confound biomarkers of iron status. [7] The relative, quantitative effects of these processes compared to iron status on iron markers are not known. Limited data regarding sensitivity and specificity of iron markers in SCD exist. Low serum ferritin is specific for iron deficiency but in SCD has low sensitivity due to effects of inflammation. [8] Transferrin saturation is decreased in iron deficiency but can also be decreased by the hypoferremic acute phase response to infection and inflammation. The gold standard method of iron staining in bone marrow biopsies is rarely conducted; but in one small study in 60 Indian SCD adult patients, 28% were reported to have absent stainable iron in bone marrow aspirates. Importantly, although the specificity of ferritin <30 ng/mL for iron deficiency by bone marrow was 99%, the sensitivity was only 32%, indicating iron deficiency may be present at higher ferritin concentrations in this condition. [8]

The severity of anemia in SCD may also be affected by the *co*-inheritance of other SCD modifying polymorphisms, including the α-thalassaemia 3·7 deletion and glucose-6-phosphate dehydrogenase (G6PD) deficiency [9]. Co-inheritance of α-thalassaemia in SCD modifies red cell indices and results in decreased hemolytic markers. [10] In some reports, it has also been associated with effects on total hemoglobin and steady-state hemoglobin level. [11] G6PD deficiency results in decreased capacity to reduce oxidized glutathione via NADPH, and thus the reduced ability of red cells to counteract oxidant stress. [10] G6PD deficiency (A-genotype) in sickle cell anemia is associated with lower hemoglobin but not increased hemolysis. [12]

Iron deficiency in non-SCD populations is associated with reduced cognitive development and function and in SCD may increase the severity of anemia, with consequent reduction in quality of life and survival. [13,14] Paradoxically, there are reports that patients with SCD in iron-deficient states may have better SCD-related clinical outcomes. [15,16] Levels of hemolysis and steady-state hemoglobin level have been used to differentiate severity of disease in SCD. [17]

Here, we sought to determine if hepcidin is associated with lower or higher average steady-state hemoglobin (ASSH) level in children with SCD; and to evaluate how such an effect might be mediated.

2. Methods

2.1. Study Design and Population

The study was conducted in children enrolled in the Muhimbili Sickle Cohort (MSC) in Dar-es-Salaam, Tanzania who regularly attended the outpatient clinic between 2006 and 2009. [14] Samples were selected for analysis from children in the lowest or highest average (over 12 months) steady-state hemoglobin (ASSH) quartile within their age group (HbQ1 or HbQ4). Selecting comparison populations based on usual hemoglobin status, rather than hemoglobin on a single day, reduced potential variability. Prospectively collected and archived steady-state plasma and serum samples were selected as per the criteria outlined below from children aged 3–18 years on the day of sample collection. At that time, routine penicillin prophylaxis and pneumococcal vaccination had not been implemented and no children were on regular blood transfusions. Venous blood for blood counts and peripheral oxygen saturation (SpO_2) using pulse oximetry (Nelcor Haywood, CA or Masimo Radical, Irvine CA, USA) were routinely collected at these clinic visits. The definition of steady-state for selection of blood samples to be assessed for the laboratory investigations and for the calculation of ASSH was defined as a routine scheduled outpatient clinic visit in the absence of recorded pain, fever (temperature > 37·4 °C), or current/recent malaria infection (malaria rapid test or slide positive). Samples were further excluded if hospitalization was known to have occurred within a month of sample collection, or were probable HbSβ$^+$. Otherwise, all individuals were HbSS diagnosed by quantification of hemoglobin fractions performed by high performance liquid chromatography (HPLC) using the β-thalassaemia Short Program on the Variant I analyser (BioRad, Hercules, CA, USA).

2.1.1. Laboratory Procedures

Blood counts (Pentra 60, Horiba ABX, Kyoto, Japan) were routinely collected on EDTA samples. Fetal hemoglobin was quantified using HPLC. At annual visits, serum samples were collected for routine clinical chemistry analyses (Roche Cobas Mira, New York or Abbott Architect, New York, USA) as part of MSC follow-up and included liver function tests; aspartate transaminase (AST), alkaline phosphatase (ALP) and direct and indirect bilirubin, plus lactate dehydrogenase (LDH) as a marker of hemolysis. Remaining plasma and serum samples were archived at −80 °C. Serum ferritin, serum iron, transferrin, C-reactive protein (CRP) and α-1 acid glycoprotein (AGP) were measured in archived samples in batches on an automated analyser (Roche Cobas Mira, New York). Plasma erythropoietin (EPO) and soluble transferrin receptor (sTfR) were measured using ELISA (R&D systems) as per manufacturer's instructions. Plasma hepcidin was measured with a competitive ELISA (Hepcidin-25 [human] EIA Kit, Bachem) by a trained laboratory scientist using an adapted method as published elsewhere. [18] Hepcidin:transferrin saturation and hepcidin: ferritin ratios were calculated as surrogate measures of hepcidin expression on circulating and stored iron respectively, as previously described [19,20]. All samples were analysed in the Muhimbili Sickle Cohort research laboratory in Muhimbili National Hospital, Tanzania. Standards and samples were analysed in duplicate or triplicate (hepcidin). Samples with readings outside the standard curvilinear region were repeated at appropriate dilutions. Sample results with a coefficient of variation over 10% (≥12% for hepcidin) were repeated.

Co-inheritance of potential SCD modifying genotypes including α–thalassaemia genotype for the 3·7 deletion and The 202- and 376-single nucleotide polymorphisms (SNPs) (rs1050828 [G-202A] & rs1050829 [A-376G]), the combined inheritance resulting in the A- phenotype of glucose 6-phosphate dehydrogenase (G6PD) deficiency were assessed as previously reported. [9]

2.1.2. Statistical Analysis

Data were analysed using Stata IC (StataCorp LP v14·1). Adjustment for within individual clustering for repeated samples was accounted for using robust standard errors. Statistical significance was set as $p \leq 0{\cdot}05$. Missing data of ≥5% per variable with a pattern of missingness that was random or completely random was imputed using chained equations and $n = 20$ imputation sets. [21]

Univariable analysis accounting for repeated observations in some individuals was performed using logistic regression. Results were reported as odds ratios with associated 95% confidence intervals.

Please cite this article as: Lee N, et al, Decreased Hepcidin Levels Are Associated with Low Steady-state Hemoglobin in Children With Sickle Cell Disease in Tanzania, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.07.024