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Haemoglobin variants, iron status and anaemia in Sri Lankan adolescents with low red cell indices: A cross sectional survey☆,☆☆

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ABSTRACT

Iron deficiency complicates the use of red cell indices to screen for carriers of haemoglobin variants in many populations.

In a cross sectional survey of 7526 secondary school students from 25 districts of Sri Lanka, 1963 (26.0%) students had low red cell indices. Iron deficiency, identified by low serum ferritin, was the major identifiable cause occurring in 550/1806 (30.5%) students. Low red cell indices occurred in iron-replete students with alpha-thalassaemia including those with single alpha-globin gene deletions. Anaemia and low red cell indices were also common in beta-thalassaemia trait. An unexpected finding was that low red cell indices occurred in 713 iron-replete students with a normal haemoglobin genotype.

It is common practice to prescribe iron supplements to individuals with low red cell indices. Since low red cell indices were a feature of all forms of α thalassaemia and also of iron deficiency, in areas where both conditions are common, such as Sri Lanka, it is imperative to differentiate between the two, to allow targeted administration of iron supplements and avoid the possible deleterious effects of increased iron availability in iron replete individuals with low red cell indices due to other causes such as α thalassaemia.

1. Introduction

The α and β thalassaemias are inherited disorders of haemoglobin, and together with sickle cell disease are the most common monogenic diseases of man, occurring at highest frequency in the tropical regions of the world [1]. It is estimated that > 7% of the world's population carry a haemoglobin variant, resulting in 300,000–500,000 babies born each year with a serious haemoglobin disorder. 90% of these births occur in low and middle-income countries. Although clinically less significant than β thalassaemia, α thalassaemia is the most common form of thalassaemia and it is estimated that approximately 5% of the world's population are carriers [1]. Phenotypes range from clinically silent carriers to the lethal condition of Hb Bart's hydrops fetalis.

More than 100 forms of α thalassaemia have been described, and they are classified as either α^+ thalassaemia in which one of the alpha globin genes on a single chromosome is deleted ($-\alpha/\ \alpha\alpha$) or α^0-thalassemia when both alpha globin genes on a single chromosome are deleted ($--/\alpha\alpha$). Common α^+ thalassaemia variants are due to 3.7 kb or 4.2 kb deletions in the α globin gene. The 3.7 kb deletion is the most common type, accounting for over 80% of cases of α thalassaemia [2–5] and is predominantly found in Africa, the Mediterranean and Asia while the 4.2 kb deletion is found in South-east Asia and the Pacific Islands. Common α^0-thalassemia variants include the $--^{MED}$, $-\ -\alpha^{20.5}$, found in the Mediterranean region and $--^{SEA}$, $--^{FIL\ and}$ $--^{THAI}$ which are most prevalent in South-east Asia [6].

We have previously reported the frequency of haemoglobin variants in an island-wide survey of > 7500 adolescent students attending schools in the 25 districts of Sri Lanka. α^+ thalassaemia, due to a 3.7 kb deletion on a single alpha globin gene ($-\alpha^{3.7}/\alpha\alpha$) was the most common haemoglobinopathy trait detected, and was present in 2.9–20% of the study population, varying according to district. α^+ thalassaemia due to a 4.2 kb deletion ($-\alpha^{4.2}/\alpha\alpha$) was less common, as

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was the form of α^0 thalassaemia previously described in Sri Lanka ($--^{SL}/\alpha\alpha$) [7].

Normal adult haemoglobin is made up of 2 alpha globin and 2 beta globin chains, each with a central heme prosthetic group. In α^+ thalassaemia there is reduced production of α globin chains. In the homozygous and compound heterozygous states, this results in low Mean Cell Volume (MCV) and/or low Mean Cell Haemoglobin (MCH). The red cell indices associated with α thalassaemia due to a single α globin gene deletion are generally considered to be normal [8–11]. We have recently found that in a survey of > 7500 Sri Lankan school students to determine the frequency of haemoglobin variants, low red cell indices were present in 1963/7526 (26%) students [12].

Low red cell indices are also a feature of iron deficiency; the most common micronutrient disorder and cause of anaemia worldwide [13]. WHO recommends the measurement of serum ferritin, transferrin receptor and haemoglobin concentrations to classify the progressive stages of iron deficiency as follows [13,14]:

- *Iron depletion*: low iron stores but physiological functions are normal: low serum ferritin.
- *Cellular iron deficiency*: Iron insufficiency is more marked, iron stores are exhausted. and cellular physiological functions are impaired: low serum ferritin and raised transferrin receptor.
- *Iron deficiency anaemia*: Iron deficiency persists long enough to reduce red cell mass: low haemoglobin (Hb) concentration, low serum ferritin and raised transferrin receptor.

We have recently reported that in Sri Lankan adolescents without haemoglobinopathy traits iron deficiency was common, particularly in the Tamil ethnic group and in females < 16 years of age [12]. To our knowledge the iron status of individuals with haemoglobinopathy traits in Sri Lanka have not been reported previously. Here we report the contribution of iron deficiency and haemoglobinopathy traits to low red cell indices in the student population.

2. Material and methods

The study population and enrolment procedure used in this cross-sectional survey have been described previously [7,12]. Briefly, Between June 2009 and July 2010, we recruited 7526 school children aged 11–19 years from 72 schools across the 25 districts of Sri Lanka. Approximately 300 students were enrolled from each district, from schools that were geographically spaced and the major ethnic groups were represented in the student population. Prior to enrolment into the study, signed, informed consent was obtained from the parents/carers of all participating students. Enrolment was voluntary and any student or parent/carer who did not wish to participate were allowed to decline. Also, any student who felt unwell on the day of the survey was not recruited into the study.

3. Laboratory procedures

Details of the laboratory methods used for the measurement of haematological indices, haemoglobin variant detection, DNA extraction, serum ferritin, transferrin receptor and iron have been described previously [7,12,15]. Briefly, 5 ml venous blood was collected from each student; 2.5 ml was transferred into a plain tube and the remaining sample to a tube containing EDTA anticoagulant.

The EDTA sample was used for the detection of haemoglobin variants by High Performance Liquid Chromatography (BioRad, India), measurement of haemoglobin, and red cell indices using a Coulter counter (Beckman Coulter, UK) and measurement of Zinc Protoporphyrin (ZPP) using a front-faced haematofluorimeter and Protofluor reagent system (Helena BioSciences, South Shields, UK).

The remaining EDTA sample was centrifuged, the buffy coat removed, DNA extracted and alpha globin genotype determined by multiplex polymerase chain reaction and sequencing as described previously [16,17].

The blood sample transferred to the plain tube was allowed to clot, centrifuged, and the serum separated and shipped to UK on dry-ice for the measurement of iron biomarkers including iron, ferritin and transferrin receptor. All biomarkers were measured in duplicate, using methods described previously [12,15].

Students were classified as having low red cell indices if they had a mean cell volume (MCV) < 80 fl and/or a mean cell haemoglobin (MCH) < 27 pg, based on recommended guidelines [18–20].

Anaemia was defined as Hb < 11.5.0 g/dl in children < 12 years, Hb < 12.0 g/dl in females ≥12 years and males aged 12–14 years and Hb < 13.0 g/dl in males aged 15 years and over. Cut-off values used to define iron deficiency were: ZPP > 70 μmol/mol heme, ferritin < 15 ng/ml, in accordance with WHO guidelines [21] and transferrin receptor > 28.1 nmol/l and serum iron < 10.6 μmol/l in males and < 6.6 μmol/l in females, in accordance with the kit manufacturers' guidelines.

4. Statistical analysis

Categorical variables were expressed as counts and percentages and compared using the chi-square test. Continuous variables were expressed as median (inter-quartile range). All data analysis was performed using Statistical Package for Social Sciences (SPSS) software, version 24.

5. Ethical approval

This study and the consent procedures were approved by The Ethical Committee, University of Kelaniya, Sri Lanka, The Sri Lankan School Authorities, The Sri Lankan Ministry of Health and Oxford University Tropical Research Committee, Oxford, UK.

6. Results

Of 7526 students, 1963 (26.1%) had low red cell indices (MCV < 80 fl and/or MCH < 27 pg). Haemoglobin concentration was measured in 1878 samples with low red cell indices, and anaemia was common, particularly in those with haemoglobinopathy traits (Table 1).

6.1. Iron deficiency

The most commonly identified cause of low red cell indices was iron deficiency (Fig. 1; Table 1). Low iron stores (serum ferritin < 15 ng/ml) were present in about 1 in 3 students with a normal haemoglobin genotype and occurred with similar frequency in α-thalassaemia and β-thalassaemia trait (19.8% and 15.5% respectively; $P = 0.35$). Cellular iron deficiency and iron deficiency anaemia were less common and also occurred in a similar proportion of students with α-thalassaemia and β-thalassaemia trait (Table 1; $P = 0.13$ for both comparisons).

The frequency of low serum iron and raised ZPP was broadly similar to that of cellular iron deficiency and iron deficiency anaemia respectively in the different haemoglobin genotype groups. The exception was β –thalassaemia trait where raised ZPP was more common than iron deficiency anaemia ($P < 0.0001$).

6.2. Haemoglobin variants

Amongst the 1256 students with normal iron status, the most commonly identified cause of low red cell indices was α^+ thalassaemia (413; 32.9%), β-thalassaemia trait (93; 7.4%) and HbE trait (18; 1.4%) with other haemoglobin variants occurring at lower frequencies (Table 1). Notably, single α-globin deletions were associated with low red cell indices in iron replete students (351/440 [79.8%] students with $-\alpha^{3.7}/\alpha\alpha$ and 42/52 [80.8%] students with $-\alpha^{4.2}/\alpha\alpha$; Table 1). In total,