SUPPLEMENTARY APPENDIX

Optimizing diagnostic biomarkers of iron deficiency anemia in community-dwelling Indian women and preschool children

Giridhar Kanuri,[1,2] Deepti Chichula,[1] Ritica Sawhney,[1] Kevin Kuriakose,[1] Sherwin De'Souza,[1] Faye Pais,[1] Karthika Arumugam[1] and Arun S. Shet[1,3]

[1]Wellcome Trust- DBT Hematology Research Unit, St. Johns Research Institute, Bangalore, Karnataka, India; [2]Department of Biotechnology, KLEF, Greenfields, Vaddeswaram, Andhra Pradesh, India and [3]National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA

 doi:10.3324/haematol.2018.193243

Received: March 13, 2018.

Accepted: August 6, 2018.

Pre-published: August 9, 2018.

Correspondence: arun.shet@nih.gov

Methods

Study location and Participants

The present study used blood samples obtained at baseline in the context of conduction of a pragmatic cluster randomized trial[1]. This trial evaluated the effectiveness of a lay health worker led educational intervention when added to usual treatment with iron in improving anaemia cure rates in 12-59 months old anaemic children. The main results of this trial will be reported elsewhere[2]. The study area was located in rural Chamarajnagar district, Karnataka, South India where malnutrition and nutritional anaemia is widespread and the prevalence of haemogobinopathy, malaria, and hookworm infestation is low[3]. From 270 villages in the study area, 60 villages were selected randomly and all healthy community-dwelling rural children aged 12-59 months (n=1144) and their mothers (n=878) living in these villages were invited to participate. Baseline sociodemographic information and blood samples from participating mothers and their children were obtained as previously described[1]. The present study also included blood samples obtained during the pilot evaluations of the trial data collection instruments (children n = 212). All participants enrolled provided informed consent and had a complete blood count performed using an automated cell counter (Sysmex XP-100, Sysmex, Japan). In the pretrial pilot study, some mothers/caregivers initially provided consent to participate which they subsequently withdrew resulting in the exclusion of their children (n=7).

Samples size

We based the sample size calculation for the trial on our primary aim to show a minimum 12% difference in anaemia cure rate between the two experimental groups (intervention and standard treatment). However, this aim is not relevant to the present study but the sample size

calculations are reported elsewhere[1]. In the present study, we included all children enrolled in the main trial and all children recruited during pilot studies for the trial.

Eligibility criteria

All children aged 12-59 months and their mothers/caregivers residing in the villages randomly selected to participate in the trial were eligible. Children were excluded in they had a history of an active infection, history of fever or a recorded temperature >101 °F during the day of recruitment or a recent blood transfusion and/or severe anaemia (haemogobin [Hb] ≤7.9 g/dL).

Sample Processing

Erythrocytes from EDTA anticoagulated blood were washed by centrifugation at 2000*g* for 15 min in equal volumes of 0.85% normal saline x2 and stored at -80°C. Erythrocytes samples were thawed and used for ZPP/H analysis. Blood samples collected in serum separation tubes were centrifuged at 1200*g* for 10 min to prepare serum and the serum was stored at -80°C. Serum samples were thawed and used for hepcidin, ferritin, CRP and sTfR assays.

References

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