Electronic Supplementary Material (ESI) for Metallomics.

SUPPORTING INFORMATION

Association of B_{12} deficiency and anemia synergistically increases the risk of high TNF-α level among adolescent girls.

N. S. Ghatpande, P. P. Apte, S. S. Naik, B. N. Joshi, M. K. Gokhale and P. P. Kulkarni

Subjects and methods

Ethical consideration: The study was approved by the ethical committee of King Edward Memorial Hospital Research Center, Pune, India. All the methods were carried out in accordance with approved guidelines. A written informed consent was obtained from guardians of all girls included in the study group.

Subjects: 101 adolescent girls age range 11-16 years studying in 'Janna Prabodhini' school in Pune, India participated in the study. All school girls had not received hormone, iron, biological agents, vitamins and blood transfusion therapy in recent one month and did not suffer from any other hematological diseases (such as thalassemia, sickle cell anemia, iron overload), infectious diseases (such as acuteinfectious enteritis, intestinal tuberculosis, intestinal amebic dysentery, inflammatory bowel disease) and malignant diseases.

Anthropometric measurements: Body weight (kg) was measured using an electronic weighing balance to the nearest 0.02 kg. Height was measured using stadiaometer to nearest 0.1 cm. Percent body fat was measured using Omron body fat monitor (Japan) which works on bio-impedance analysis principle. All readings were taken in duplicates by a trained investigator and the average of two was used for analysis. Body mass index (BMI) (kg m^{-2}) was calculated as weight (kg) divided by the square of height (m).

Biochemical estimations: Blood samples were collected by a trained technician in 5 mL anticoagulant tubes (K_2EDTA Labtech Disposables). Hb (g dL^{-1}) was measured using Lablife Nobel III automated hematology analyzer (Diagnova, India). Aliquots of blood were made in cryo vials was allowed to clot at room temperature (25°C) after which the serum was separated by centrifugation and stored at – 80°C and later used for various biochemical estimation. Multiple indicators for iron status were assessed that included serum iron (SI), Total Iron Binding Capacity (TIBC) and serum ferritin (SF). SI (µg dL^{-1}) and TIBC (µg dL^{-1}) was measured using the ferrozine colorimetric method, SF (µg L^{-1}) was measured using ELISA kit (Diagnostic Biochem, Canada). Serum hepcidin (pg mL^{-1}) (USCN life sciences, China), and TNF-α(pg mL^{-1}) was estimated using ELISA kits (Krishgen Biosystems, India). Total serum B_{12} (pg mL^{-1}) was estimated using chemiluminescent enzyme immunoassay by automated analyzer (Axsym, Abbott, USA). Current assay for total serum B_{12} measure all form of cobalamin i.e. hydroxocobalamin, adenosylcobalamin and methylcobalamin. Total serum homocysteine (tHcy, umolL^{-1}) was measured using chemiluminescent enzyme immunoassay by automated analyzer (Roche Diagnostics, USA).

Cutoffs for defining deficiency: We defined underweight in adolescent girls as BMI<18.5 kg m^{-2} and normal weight as BMI≥18.5 kg m^{-2}. Anemia was defined as Hb<12 g dL^{-1} in accordance with WHO criteria.[1] Iron deficiency (ID) was defined as SF<15 µg L^{-1} and iron deficiency anemia (IDA) was defined as Hb<12 g dL^{-1} and SF<15 µg L^{-1}.[1] B_{12} deficiency was defined as serum B_{12} concentration <200 pg mL^{-1}.[2]

Statistical analysis: All statistical analyses were performed by using Statistical Package for Social Sciences (SPSS for windows version 17.0, SPSS Inc., Chicago, US) and XLSTAT for Windows (Adinosoft, USA). Since many variables did not meet assumptions of normality, nonparametric statistics were employed. Therefore descriptive statistics were reported in terms of medians, unless specifically mentioned and interquartile range (IQR) for continuous variables and in terms of absolute frequencies and percentages for categorical variables. Spearman's rank correlation testing (r_s) was used for correlation analysis. When comparing ordinal data across categorical variables, the Mann–Whitney U test was used for categorical variables with two groups. Nonparametric receiver operating characteristic (ROC) curve were drawn, and area under the curve (AUC) values were calculated. Sensitivities and specificities of selected cutoffs were evaluated. Logistic regression analysis was used to estimate association between anemia, B_{12} deficiency and TNF-α.

References:

1. WHO, UNICEF, UNU, Iron deficiency anaemia: assessment, prevention, and control. A guide for programme managers, Geneva, WHO, 2001.
2. L. H. Allen, J. L. Rosado, J. E. Casterline, P. López, E. Muñoz, O. P.Garcia and H. Martinez, *Am. J. Clin. Nutr.*, 2000, **71**, 1485-1494.

Table S1: Anthropometric characteristics, iron status and inflammatory status in adolescent girls (n = 101).

Parameters	Median(IQR)
Anthropometric parameters:	
height (cm)	154.35(149.00-158.25)
weight (kg)	43.8(38.10-50.00)
BF (%)	23.5(17.80-29.10)
BMI (kg m^{-2})	18.25(16.10-21.13)
Iron status parameters:	
Hb(g dL^{-1})	12.5(11.80-13.10)
SI(µg dL^{-1})	58.0(32.50-82.50)
SF(µg L^{-1})	12.0(8.55-24.50)
TIBC(µg dL^{-1})	318.0(280.0-365.00)
TS (%)	17.75(8.63-28.62)
B_{12} (pg mL^{-1})	285.0(218.00-371.50)
Inflammatory status parameters:	
Hepcidin (pg mL^{-1})	80.0(58.0-158.50)
TNF-α (pg mL^{-1})	7.50(5.0-22.75)

BF: Body fat, BMI: Body mass index, Hb: Hemoglobin, SI: Serum iron, SF: Serum ferritin, TIBC: Total iron binding capacity, TS: Transferrin saturation, TNF-α: Tumor necrosis factor-alpha. IQR= Inter Quartile Range

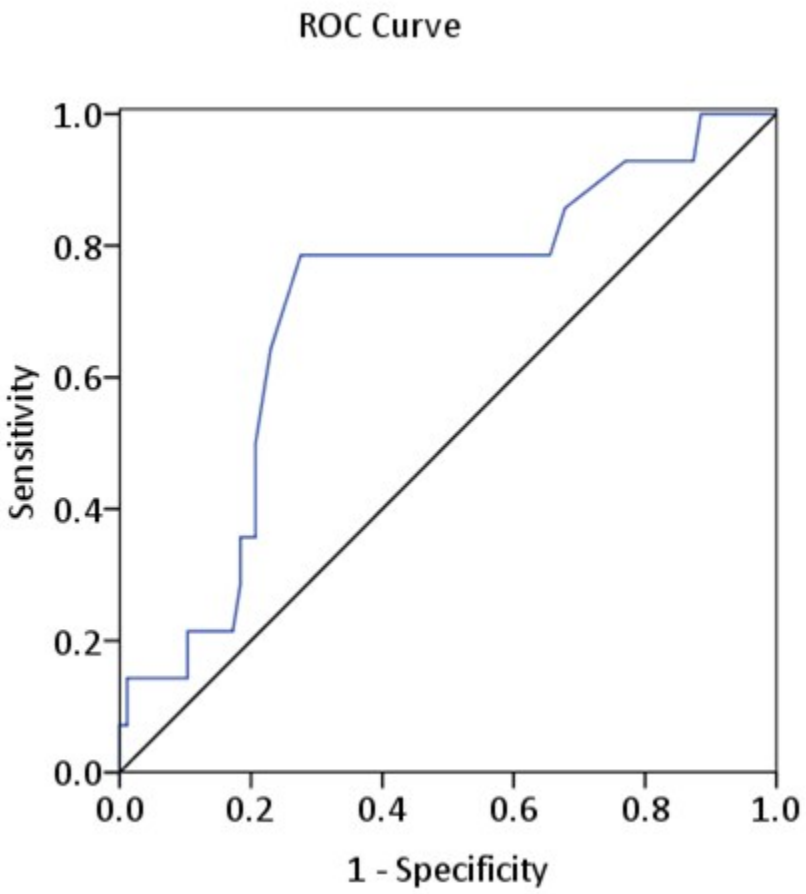

Fig. S1. ROC curve for the ability of TNF-α to distinguish B_{12} deficiency levels among 101 adolescent girls. The AUC for TNF-α to identify B_{12} deficiency among adolescent girls was 0.71 (95%CI: 0.559, 0.855).