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Anaemia, iron deficiency and vitamin A status among school-aged children in rural Kazakhstan

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Abstract

Objectives: To investigate the prevalence of anaemia and iron deficiency and vitamin A status among school-aged children in rural Kazakhstan and identify factors associated with anaemia in this population.
Design: A cross-sectional design.
Setting: School-aged children in rural Kazakhstan.
Subjects: Socio-economic and anthropometric information was collected from 159 school-aged children living in the Kzyl-Orda region of Kazakhstan. Blood samples were collected and the concentrations of haemoglobin (Hb), serum iron, serum ferritin (SF), erythrocyte protoporphyrin (EP), serum retinol and β-carotene, total iron binding capacity (TIBC), transferrin saturation (TS) and other haematological indices were measured.
Results: Among the 159 children, the prevalence of anaemia and iron deficiency defined by the multiple criteria model (SF, TS and EP) was 27% and 13%, respectively. Nine per cent had iron-deficiency anaemia and 21% had serum retinol value $<1.05\,\mu mol\,l^{-1}$. Mean SF and serum iron concentrations and TS were significantly lower in anaemic children than in their non-anaemic peers, while TIBC and EP were significantly higher in children with anaemia. Hb was significantly correlated with serum iron and retinol concentrations. Serum retinol and SF concentrations and mean corpuscular volume were significantly correlated with Hb by multiple regression analysis.
Conclusions: Anaemia among school-aged children in rural Kazakhstan appears to be related to iron indices and vitamin A status.

Keywords
Anaemia
Iron deficiency
Vitamin A
Serum retinol
School-aged children
Kazakhstan

Iron-deficiency anaemia is a major nutritional problem throughout the world and leads to serious health problems, such as poor cognitive and motor development and behavioural problems, in children[1]. The Demographic and Health Surveys in Kazakhstan[2] reported that 69% of children younger than 3 years were anaemic. However, information to examine the aetiology of anaemia is very limited in Kazakhstan and other Central Asian republics[3]. In a previous study in rural Kazakhstan, we observed a positive correlation between serum ferritin (SF) and haemoglobin (Hb) concentrations, suggesting that a significant proportion of anaemia cases might be related to iron deficiency[4]. Another study showed that higher iron intake is associated with a decreased prevalence of anaemia[5]. However, only one-third of the incidence of anaemia in this population could be attributed to iron deficiency[4] and it is possible that other important factors influence the prevalence of anaemia in the region.

Many nutritional surveys from around the world have shown a close association between vitamin A deficiency

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and anaemia[6,7]. There is clear evidence of an association between serum retinol and iron indicators[8–10], and vitamin A deficiency is considered among the causes of anaemia[11]. Vitamin A is thought to influence anaemia by modulating erythropoiesis and iron metabolism and by enhancing immunity to infection and the anaemia of infection[11]. The effect of poor vitamin A status on iron-deficiency anaemia could have widespread implications for current preventive public health interventions[11]. However, there is very limited information on vitamin A status in Kazakhstan and other Central Asian republics. In the present paper we report on a community-based study assessing the prevalence of anaemia and iron deficiency, and vitamin A status, among school-aged children in rural Kazakhstan. We also explored various factors associated with anaemia in this population.

Subjects and methods

Subjects

This study was conducted as part of a follow-up study to assess the incidence of anaemia in school-aged children 2 years after a baseline study. Detailed descriptions of the study site and the sampling procedures of the baseline study appear elsewhere[4]. Briefly, equal numbers of boys and girls born between 1985 and 1993 inclusive (8–17 years old at the time of the study) were randomly selected according to birth year from the list of children in nine villages in the Kazalinsk district of the Kzyl-Orda region. From 380 subjects enrolled in the larger baseline study, we selected all 208 participants living in five villages in the Kazalinsk district. The villages were selected as representative of the geographical distribution of villages in the district. Mothers of eligible children were invited to bring their children to the health centre on a designated day. A total of 164 children were enrolled in the study, giving a response rate of 79%. A complete set of haematological and anthropometric data was obtained from 159 children. The research protocol was reviewed and approved by the institutional ethical committee of Juntendo University School of Medicine and the Ministry of Health in Kzyl-Orda region. Children participating in the study and their parents were well informed about the research and the parents gave their written consent.

Questionnaire

A questionnaire was developed to obtain information on the family's socio-economic and demographic status such as household size, income and possessions, parents' education and the vegetables and fruits grown in the home garden at the time of interviews. Number of varieties of vegetables and fruits grown in the home garden was calculated by summing the vegetables and fruits grown in the home garden. Local nurses of public hospitals interviewed the children's mothers or guardians at the health centres using the questionnaire written in the Kazakh language.

Anthropometry

Anthropometric measurement was based on the standardised method of the World Health Organization (WHO) and the United Nations Children's Fund[12,13]. The weight of children, wearing minimal clothing, was measured to the nearest 0.1 kg on a battery-powered digital scale. Height was measured to the nearest 0.1 cm with a scale with a movable bar and steel tape. Height-for-age and weight-for-age were expressed in *Z*-scores and calculated with Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA, USA) with use of the National Center for Health Statistics reference data[14]. Stunting was defined following the WHO definition[15] as height-for-age *Z*-score below −2. Body mass index (BMI; weight in kg divided by the square of height in m) less than the 5th percentile according to the sex- and race-specific tables of Must *et al.*[16,17] was used to define wasting.

Blood sampling and biochemical measurements

A blood sample (7 ml) was collected by venepuncture of an antecubital vein. One millilitre was drawn into a container with ethylenediaminetetraacetic acid to measure white blood cell (WBC) and red blood cell (RBC) counts, Hb concentration, haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Assays were performed immediately after blood sampling using an automated cell counter (MEK-5207 Hematology Analyzer; Nihon Kohden, Tokyo, Japan). Another 1 ml of blood was placed in a tube containing heparin and wrapped with foil to protect against photodecomposition of protoporphyrins. The remaining blood was centrifuged at 3000 ***g*** for 5 min at room temperature. The sera and blood specimens were immediately frozen at −10°C, kept for 1–3 weeks and then transported to Tokyo in a portable ice box filled with solid carbon dioxide. Then all specimens were kept frozen at −80°C until analyses.

Serum retinol and β-carotene levels were determined simultaneously using high-performance liquid chromatography (HPLC). The procedure followed the method of Miller and Yang[18] with slight modification. Retinol and β-carotene were extracted with hexane after deproteinisation with ethanol containing retinyl acetate as the internal standard, and evaporated to dryness under nitrogen gas. The residue was dissolved in 1.0 ml ethanol. A portion (50 μl) of the sample was injected into the column (Shim Pack CLC-ODS, 6.0 mm × 150 mm) installed with the HPLC apparatus (LC-VP Series; Shimadzu, Kyoto, Japan). The mobile phase was a methanol–chloroform mixture (87:15). Concentrations of retinol and β-carotene were determined by spectrophotometry (SPD-10AV instrument; Shimadzu) at 325 nm for retinol and 453 nm for

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β-carotene. The intra-assay coefficients of variation for retinol and β-carotene were 0.6% and 2.7%, respectively.

Erythrocyte protoporphyrin (EP) concentrations were determined according to the previously reported method[19]. Protoporphyrins in 50 μl whole blood were extracted into 2.5 ml *N*,*N*-dimethylformamide. After centrifugation at 3000 rev min^{-1} for 5 min, 20 μl supernatant was injected into the column (JASCO Fine Pak (C18) 4.6 mm × 150 mm; pre-column Biofine 4.6 mm × 10 mm; JASCO, Tokyo Japan) of an HPLC apparatus (RF-535; Shimadzu). The mobile phase was mixture of acetonitrile–acetic acid–200 mM ammonium acetate (90:5:5) at pH 5.2. Zinc protoporphyrin and free protoporphyrin were determined separately; emission wavelength was 590 nm for zinc protoporphyrin and 630 nm for free protoporphyrin (Hitachi 320 Spectrofluorometer, Tokyo, Japan). Total free EP concentrations were calculated as free protoporphyrin concentration + zinc protoporphyrin concentration/1.1.

SF concentration was determined by the electrochemiluminescence immunoassay method enhanced with magnetic capture on an Elecsys 2010 Immunoassay Analyser (Hitachi High-Technology Corporation, Tokyo, Japan). The intra-assay coefficient of variation for SF was 4.8%. Transferrin saturation (TS) was determined by dividing the serum iron concentration by the total iron-binding capacity (TIBC) as assessed by a colorimetric procedure[20]. C-reactive protein (CRP) concentration was measured by using a latex turbidimetric immunoassay method with a commercial kit (CRP-Latex Seiken; Denka Seiken Co., Ltd, Tokyo, Japan). The intra-assay coefficient of variation for CRP was 1.2%. The laboratory personnel who measured EP and serum retinol and β-carotene concentrations were not aware of the anaemic status of study participants.

On the basis of a multiple criteria model, iron deficiency was defined when abnormal results were found for two or more of the three tests: SF, TS and EP. Values were considered abnormal if $<12\ \mu g\ dl^{-1}$ for SF, <16% for TS and $>70\ \mu g\ dl^{-1}$ for EP[20]. Iron-deficiency anaemia was defined as iron deficiency concurrent with anaemia. Anaemia was defined as Hb concentration below the cut-off for age and sex as defined by WHO ($12\ g\ dl^{-1}$ for females and 8–14-year-old males and $13\ g\ dl^{-1}$ for 15–17-year-old males)[21]. CRP concentration and WBC count were used as indicators of the presence of a possible infection or inflammation. CRP concentration $>5.0\ mg\ l^{-1}$ and WBC count $>10\,000/mm^3$ were considered abnormal.

Statistical analysis

The distribution of each set of data was tested for normality before analysis, using the Kormogorov–Smirnov goodness-of-fit test. Where necessary, data were normalised using natural-log transformations. Means, standard deviations (SD) and medians were calculated for each variable. Chi-square tests were used to compare categorical variables between groups. Differences in continuous variables between groups were examined by using Student's *t*-test. Pearson's correlation test was performed to examine the association between concentrations of serum retinol and β-carotene and measures of iron status. Backward-stepwise multiple regression analysis was performed to assess the independent relationship of Hb concentration with various socio-economic and biochemical factors. Statistical significance was set at $P < 0.05$. SPSS for Windows version 10.0 (SPSS Inc., Chicago, IL, USA, 1999) was used for statistical analysis.

Results

Socio-economic and demographic characteristics of the subjects are presented in Table 1. The sample comprised approximately equal numbers of boys and girls between the ages of 8 and 17 years inclusive. All the children were Muslim and Kazakh in origin. The percentage of parents who did not complete primary or secondary education (10 years of school) was low (5%). Despite this high education level, parents' unemployment rate was high. The median monthly household income (including

Table 1 Socio-economic and demographic characteristics of school-aged children in rural Kazakhstan ($n = 159$)

Variable	*n*	(%)
Sex		
Boy	83	(52.2)
Girl	76	(47.8)
Age group (years)		
8–10	47	(29.6)
11–13	56	(35.2)
14–17	56	(35.2)
Household size (persons)		
Up to 4	13	(8.2)
5–7	112	(70.4)
Over 7	34	(21.4)
Mother's education		
Primary/secondary incomplete (0–9 years)	8	(5.0)
Primary/secondary complete (10 years)	90	(56.6)
High (>10 years)	61	(38.4)
Income and employment		
Total income (Tenge)†	7500	(6750)
Father unemployed	47	(29.6)
Vegetables and fruits planted in home garden ($n = 115$)		
Melons	85	(73.9)
Cucumbers	84	(73.0)
Tomatoes	82	(71.3)
Onions	75	(65.2)
Watermelons	67	(58.3)
Carrots	65	(56.5)
Potatoes	52	(45.2)
Grapes	22	(19.1)
Sweet peppers	20	(17.4)
Apples	14	(12.2)
Aubergines	13	(11.3)
Berries	11	(9.6)
Nuts, seeds	0	(0.0)
Other fresh fruits or vegetables	57	(49.6)

† Values are median (1st–3rd quartile). US$1 was equivalent to 141 Tenges.

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government benefits) was 7500 Tenge, equivalent to US$53 (August 2002).

The anthropometric and biochemical indices included in the nutritional assessment are presented in Table 2. Of all children in the study, 11% exhibited stunted growth and 15% were wasted. There was no difference in the prevalence of stunting between age groups, but the prevalence of wasting was significantly different between age groups (26% for 8–10-year-olds, 20% for 11–13-year-olds and 0% for 14–17-year-olds; $P < 0.001$).

Mean (±SD) Hb concentration was 12.6 ± 1.0 g dl^{-1} in boys and 12.3 ± 1.5 g dl^{-1} in girls. The prevalence of anaemia was 27%. Age group-specific anaemia rates were 15% for 8–10-year-olds, 23% for 11–13-year-olds and 41% for 14–17-year-olds ($P = 0.009$). Using a cut-off value of 12 μg l^{-1} for SF concentration, 16% of the children were iron-depleted, while TS was decreased (<16%) in 37% of the children. In contrast, only one subject had an elevated EP concentration (>70 μg dl^{-1}). There were no differences in the prevalence of abnormal values of these iron-status indices between boys and girls. The prevalence of low SF values was significantly different between age groups (2% for 8–10-year-olds, 18% for 11–13-year-olds and 25% for 14–17-year-olds; $P = 0.006$). The prevalence of iron deficiency, based on the multiple criteria model, was 13%, and the prevalence of iron-deficiency anaemia was 9%. Among the children with normal Hb levels, 5% were iron-deficient.

The mean serum retinol concentration was 1.4 μmol l^{-1}. Twenty-one per cent of the children had serum retinol values <1.05 μmol l^{-1} and only one child had a serum retinol value <0.70 μmol l^{-1}. The median β-carotene concentration was 18.4 μg dl^{-1}.

Thirteen subjects (8%) had high CRP concentration (>5.0 mg l^{-1}) or WBC count (>10 000/mm^3). They were excluded from the statistical analysis of the relationship between vitamin A status and iron indices, because acute infection or inflammatory processes may influence these relationships[22,23].

The relationships between anaemia, iron status and vitamin A indices are shown in Table 3. Children were divided into two groups based on the presence or absence of anaemia. Serum retinol and β-carotene concentrations were not associated with anaemic status. Some evidence suggested that the proportion with serum retinol concentration <1.05 μmol l^{-1} was higher in children with anaemia

Table 2 Anthropometric and haematological indices of school-aged children in rural Kazakhstan ($n = 159$)

	Boys ($n = 83$)			Girls ($n = 76$)		
Variable	Mean	SD	Median	Mean	SD	Median
Anthropometry						
Height-for-age *Z*-score	−0.97	0.91	−0.91	−0.96	0.91	−0.98
Weight-for-age *Z*-score	−0.78	0.78	−0.84	−0.89	0.84	−1.04
Body mass index (kg m^{-2})	17.8	2.0	17.7	17.7	3.2	17.2
Stunting, *n* (%)†		11 (12.8)			6 (7.9)	
Wasting, *n* (%)‡		9 (10.5)			14 (18.4)	
Blood analysis						
Red blood cells (× 10^4/mm^3)	503	43	496	488	42	485
Haemoglobin (g dl^{-1})	12.6	1.0	12.7	12.3	1.5	12.6
Haematocrit (%)	44.7	4.1	44.0	43.8	4.7	44.6
Mean corpuscular volume (fl)	88.9	5.2	90.0	89.9	8.0	90.0
Mean corpuscular haemoglobin (pg)	25.2	2.0	25.4	25.2	2.9	25.5
Mean corpuscular haemoglobin concentration (g dl^{-1})	28.3	1.4	28.3	28.0	1.5	28.1
Serum ferritin (μg l^{-1})	33.7	20.0	30.0	33.5	46.9	25.5
Serum iron (μg dl^{-1})	69.0	29.0	70.0	67.0	36.3	66.5
Serum total iron-binding capacity (μg dl^{-1})	362.3	54.2	356.0	380.3	52.5	377.0
Transferrin saturation (%)	19.6	8.3	20.3	18.4	11.4	17.8
Erythrocyte protoporphyrin (μg dl^{-1})	18.0	8.5	15.6	19.6	12.9	16.1
Serum retinol (μmol^{-1})	1.4	0.3	1.3	1.4	0.4	1.3
Serum β-carotene (μg dl^{-1})	20.7	11.1	18.4	23.0	14.9	18.5
Anaemia, *n* (%)§						
Mild		21 (25.3)			14 (18.4)	
Moderate		2 (2.4)			4 (5.3)	
Severe		0 (0.0)			2 (2.6)	
Iron deficiency, *n* (%)¶		7 (8.4)			13 (17.1)	
Vitamin A deficiency, *n* (%)						
Serum retinol <1.05 μmol l^{-1}		15 (18.1)			18 (23.7)	
Serum retinol <0.70 μmol l^{-1}		0 (0.0)			1 (1.3)	

SD – standard deviation.
† Height-for-age *Z*-score < −2.
‡ Body mass index below the 5th percentile of the reference population[16,17].
§ Mild anaemia – haemoglobin (Hb) concentration above 10.0 g dl^{-1} but below 12.0 g dl^{-1} for females and 8–14-year-old males or above 10.0 g dl^{-1} but below 13.0 g dl^{-1} for 15–17-year-old males; moderate anaemia – Hb between 7.0 and 10.0 g dl^{-1}; severe anaemia – Hb below 7.0 g dl^{-1}.
¶ Abnormal values for two or more of three iron-status indicators: ferritin (<12 μg dl^{-1}), transferrin saturation (<16%) and erythrocyte protoporphyrin (>70 μg dl^{-1}).

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Table 3 Mean values for biochemical indices by anaemic status of school-aged children in rural Kazakhstan ($n = 146$)

Variable	Anaemic† ($n = 40$)		Non-anaemic ($n = 106$)		*P*-value
	Mean	SD	Mean	SD	
Haematocrit (%)	40.2	4.4	45.8	3.4	<0.001
Mean corpuscular volume (fl)	85.8	9.1	90.7	5.2	<0.001
Mean corpuscular haemoglobin (pg)	23.6	3.4	25.8	1.7	<0.001
Mean corpuscular haemoglobin concentration ($g\,dl^{-1}$)	27.3	1.6	28.5	1.3	<0.001
Serum ferritin ($\mu g\,l^{-1}$)	20.8	19.8	34.7	18.9	<0.001‡
Serum iron ($\mu g\,dl^{-1}$)	52.8	30.0	74.1	24.9	<0.001
Serum total iron-binding capacity ($\mu g\,dl^{-1}$)	392.6	63.7	361.2	45.8	0.001
Transferrin saturation (%)	14.4	9.0	20.9	7.3	<0.001
Erythrocyte protoporphyrin ($\mu g\,dl^{-1}$)	25.4	16.3	16.3	6.5	<0.001‡
Serum retinol ($\mu mol\,l^{-1}$)	1.3	0.4	1.4	0.3	0.111
Serum retinol concentration $<1.05\,\mu mol\,l^{-1}$, *n* (%)	12 (30.0)		17 (16.0)		0.059§
Serum β-carotene ($\mu g\,dl^{-1}$)	21.3	12.1	22.3	13.6	0.716‡

SD – standard deviation.
† Anaemia – Hb concentration below $12.0\,g\,dl^{-1}$ for females and 8–14-year-old males or below $13.0\,g\,dl^{-1}$ for 15–17-year-old males.
‡ Based on natural log-transformed values.
§ Pearson chi-square test.

than in non-anaemic children ($P = 0.059$). Mean Hct, MCV, MCH, MCHC, TS and SF and serum iron concentrations were significantly lower in children with anaemia than in their non-anaemic peers. Serum TIBC and EP concentrations were significantly higher in children with anaemia.

Table 4 shows the partial correlation coefficients between different measures of Hb and vitamin A and iron-status indices adjusted for the effects of age, sex, BMI, household per capita income, years of parents' education, varieties of vegetables and fruits grown in the home garden and iron supplementation. Serum retinol concentration was significantly positively correlated with concentrations of serum iron and Hb. There was a weak, negative correlation between serum retinol and EP concentrations, whereas β-carotene concentration was not significantly correlated with serum retinol or Hb levels or iron-status indices. Hb concentration was highly correlated with SF, serum iron and EP concentrations, TIBC and TS.

Backward-stepwise multiple regression analysis (Table 5) was used to identify the factors influencing Hb levels. The analysis included the variables age, sex, BMI, household per capita income, years of parents' education, varieties of vegetables and fruits grown in the home garden, iron supplementation, MCV, TS and concentrations of serum ferritin, EP, serum retinol and β-carotene. Using a *P*-value >0.10 for exclusion, serum retinol and SF concentrations and MCV were significantly related to Hb level. TS was weakly related to Hb concentration ($0.05 < P < 0.10$). The overall *F*-ratio for all variables was 48.9 (df = 4) and was highly significant ($P < 0.001$).

Discussion

We explored the prevalence of iron deficiency and anaemia, and vitamin A status, among school-aged children in rural Kazakhstan. To our knowledge, the relationships between iron deficiency, vitamin A deficiency and anaemia have not been well characterised among school-aged children. Our study is the first to report on vitamin A status in Kazakhstan and also on iron status based on the multiple criteria model in Kazakhstani children.

There are three stages in the development of iron-deficiency anaemia[20]. The first stage is characterised by depletion of iron stores as reflected by a decline in SF concentration. The second phase, iron-deficient

Table 4 Partial correlation coefficients† for vitamin A and iron indices of school-aged children in rural Kazakhstan ($n = 146$)

Variable	SF‡	Serum Fe	Serum TIBC	TS	EP‡	Serum retinol	Serum β-carotene‡
Hb	0.644***	0.559***	−0.323***	0.529***	−0.487***	0.179**	−0.056
SF	–	0.477***	−0.599***	0.550***	−0.498***	0.024	−0.017
Serum Fe	–	–	−0.312***	0.956***	−0.450***	0.173**	0.047
Serum TIBC	–	–	–	−0.535***	0.490***	0.115	−0.005
TS	–	–	–	–	−0.495***	0.113	0.059
EP	–	–	–	–	–	−0.162*	0.129
Serum retinol	–	–	–	–	–	–	0.062

SF – serum ferritin; TIBC – total iron-binding capacity; TS – transferrin saturation; EP – erythrocyte protoporphyrin; Hb – haemoglobin.
† Coefficient adjusted for age, sex, body mass index, household per capita income, years of parent's education, variety of vegetables and fruits grown in the home garden and iron supplementation.
‡ Based on natural log-transformed values.
*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.01$.

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Table 5 Backward-stepwise multiple regression for haemoglobin concentration of school-aged children in rural Kazakhstan ($n = 146$)

Variable	*B*	SE *B*	Beta	95% CI for *B*	*P*-value
Mean corpuscular volume (fl)	0.078	0.013	0.405	(0.053, 0.102)	<0.001
Serum ferritin† ($\mu g\,l^{-1}$)	0.617	0.119	0.364	(0.381, 0.852)	<0.001
Serum retinol ($\mu mol\,l^{-1}$)	0.545	0.221	0.137	(0.109, 0.981)	0.015
Serum transferrin saturation (%)	0.020	0.011	0.127	(−0.002, 0.043)	0.071

B – ordinary least-squares regression coefficient; SE *B* – standard error of *B*; Beta – standardised β coefficient; CI – confidence interval.
Model summary: multiple $R = 0.767$; $R^2 = 0.588$; adjusted $R^2 = 0.576$; *F*-ratio = 48.9 (df = 4); $P < 0.001$.
† Based on natural log-transformed values.

erythropoiesis, is characterised by a decrease in TS and an increase in EP concentration. The final stage of iron deficiency is characterised by a reduction in the concentration of Hb in RBCs. The use of multiple indices of iron status provides a more accurate measure of iron status than any single index[24].

In our study, 16% of the children had depleted iron stores (SF $<12\,\mu g\,l^{-1}$), 37% exhibited iron-deficient erythropoiesis as defined by a reduction of TS (<16%) and only one subject (0.6%) had an elevated EP concentration ($>70\,\mu g\,dl^{-1}$). Because SF concentration is also elevated during infection or inflammation, some of our values could be false-negative results[23]. However, excluding those with elevated CRP concentration ($>5.0\,mg\,l^{-1}$) or WBC count ($>10\,000/mm^3$) from the analysis gave a similar percentage (16%) of the children with iron-storage depletion. Fewer children had high EP concentration than had high TS. EP concentration can be in the normal range independent of iron deficiency (e.g. in thalassaemia)[20,25]. The proximity of the study site to the Caucasus region and Turkey, where the genetic condition of thalassaemia contributes to a high prevalence of anaemia[26], warrants further study to determine whether a genetic predisposition for thalassaemia in Kazakhstani children may partly explain this discrepancy.

In our study, 27% of the children were anaemic and 9% had iron-deficiency anaemia. Our findings suggest that iron-deficiency anaemia is a public health problem among school-aged children in the area. However, using the strict criterion of abnormal results for two or more of the three tests (SF, TS and EP), we identified iron-deficiency anaemia in only one-third of all anaemic children, which was far less than we expected. When iron-deficiency anaemia was defined as anaemia plus low SF concentration, which might be more sensitive in detecting early depletion of body iron stores, the percentage increased slightly to 37% of all anaemic children. However, more than 60% of the cases with anaemia were not classified as iron-deficient.

We used multivariate analysis to determine whether iron status differed between anaemic and non-anaemic children. Anaemic children had significantly lower Hct, MCV, MCH, MCHC, TS, and SF and serum iron concentrations, and higher serum TIBC and EP concentrations, compared with non-anaemic children, suggesting that iron status was likely to be an important determinant of Hb concentration and anaemia.

Our study showed that 21% of children had inadequate serum retinol values ($<1.05\,\mu mol\,l^{-1}$) and indicated significant correlations between concentrations of serum retinol and Hb and serum iron, indicating a possible relationship between vitamin A status and the use of iron for haematopoiesis. A positive correlation between Hb and serum or plasma retinol concentrations has been described in children in Guatemala[8], Thailand[7], Central America[6], Pakistan[27], Bangladesh[9,28], India[29], Indonesia[30] and Ethiopia[31]. Lower serum retinol concentration has also been associated with lower iron indices. For example, Ahmed *et al.*[9] reported significant correlations between the concentrations of serum retinol, serum iron and Hb, packed cell volume, MCHC and TS in adolescent girls in urban Bangladesh. Similar observations have also been reported in children less than 5 years of age[8] and in school-aged children[6].

The multiple regression analysis revealed that Hb concentration was independently associated with MCV and SF and serum retinol concentrations. There was a weak relationship between Hb concentration and TS. Accounting for other factors showed that for every $1\,\mu mol\,l^{-1}$ change in serum retinol concentration there is a $0.5\,g\,dl^{-1}$ change in Hb level. This effect is half of that reported by Ahmed *et al.*[10], who found a $1.0\,g\,dl^{-1}$ change in Hb level in adolescent girls in Bangladesh. Our results may differ because we included both boys and girls and different age groups, and possibly because of residual confounding.

Because vitamin A is required for the effective utilisation of iron and for maintaining normal Hb concentration[8,32–36], the success of iron fortification or supplementation programmes is likely to vary according to vitamin A status. In 2001, Kazakhstan developed national plans for universal iron fortification of wheat flour[37]. Although this approach can be useful for promoting the anaemia prevention and control programme in Kazakhstan, our data reinforce the suggestion that programmes designed to reduce anaemia should also aim to improve vitamin A status.

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Conclusions

Our results suggest that anaemia among school-aged children in rural Kazakhstan cannot be explained solely by iron deficiency. Anaemia was independently related to vitamin A status as well. Programmes designed to reduce anaemia should also aim to improve vitamin A status in rural Kazakhstan.

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