Incidence of iron-deficiency anaemia in infants in a prospective study in Jordan

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Abstract: A high prevalence of iron-deficiency anaemia has been reported in Jordanian infants. A prospective study of infants in downtown Amman examined the relationship between anaemia in pregnancy and iron deficiency in infancy. The iron status of infants born to 107 anaemic (Hb <11 g/dl) and 125 non-anaemic mothers was reviewed at 3, 6, 9 and 12 months. Indicators to define iron-deficiency anaemia were Hb <11 g/dl and either plasma ferritin <12 μg/l or zinc protoporphyrin (ZPP) >35 μg/dl whole blood. Haemoglobin electrophoresis excluded haemoglobinopathy. There was 72% iron-deficiency anaemia throughout the year, significantly higher in infants born to anaemic mothers (81%; $n=91$) compared with controls (65%; $n=112$). At 12 months, 72% of the infants tested ($n=195$) were anaemic. While 57% were identified as iron-deficient by research criteria of either ferritin or ZPP, only 37% were identified by ferritin alone, 40% by ZPP alone and 29% if both ferritin and ZPP were required to meet criteria. Most infant anaemia was identified as due to iron deficiency, supporting contextual setting as assisting diagnosis: infants in developing countries are recognised as vulnerable to iron deficiency. Using multiple criteria, more cases were identified when either ferritin or ZPP were abnormal than when one alone, or both parameters were required to meet research criteria.

Key words: anaemia; iron deficiency; infancy; ferritin; zinc protoporphyrin

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Iron deficiency remains the most prevalent nutritional deficiency world-wide, the most vulnerable groups affected being women of child-bearing age, infants and young children (1, 2). Iron deficiency in infancy has been shown to delay irreversibly cognitive and psychomotor development (3–5), even after correction of the anaemia (3). Adverse effects were noted when infant haemoglobin was below Hb 11 g/dl, and increased as anaemia became more severe or chronic (3, 4). Follow-up at 5 and 10 yr indicated long-term impairment (4, 5).

In 1990, the United Nations Relief and Works Agency for Palestinian Refugees in the Near East (UNRWA) (6) reported maternal and infant iron-deficiency anaemia in 50–65% of the Palestinian population for whom they provide health care, with the highest prevalence in infants 6–12 months of age. Anaemia, defined by a haematocrit of <33%, was prevalent in the vulnerable groups, women and children but not in the male population (7), in comparison with a reference population in the United States (USA). An iron supplementation programme was begun in the UNRWA clinics with positive local results.

There is no universal definition of iron-deficiency anaemia, making comparison of research findings difficult. While the UNRWA report (6) defined the high prevalence of anaemia in the Palestinian infants in Jordan as due to iron deficiency, other studies have used various criteria to define iron deficiency (8–17). A prevalence of 9% iron deficiency and 3% iron-deficiency anaemia was reported for infants 1–2 yr of age in the USA, based on a nationally representative sample for 1988–1994 (15). Iron deficiency was defined by an abnormal value for at least 2 of 3 laboratory tests for iron [illegible] saturation and serum ferritin. An arbitrary lower reference level for haemoglobin to define anaemia has been used in studies, together with varying combinations of iron status indicators, plasma ferritin, transferrin saturation, zinc protoporphyrin (ZPP), transferrin receptor or a microcytic, hypochromic blood film. Some authors have reported anaemia in infants where other indicators did not confirm iron deficiency (16). Anaemia may reflect other nutritional deficiencies, or haemoglobinopathy, and a strict use of several indicators to define iron deficiency may fail to identify some cases (7, 16).

Our study compared the incidence of iron-deficiency anaemia during the first year of life in infants born to anaemic and non-anaemic mothers (17) and aims to evaluate the haematological variables used.

Method

Study population

A prospective case-control study of infants from birth to 1 yr was conducted (October 1993 to June 1996) at a Ministry of Health Maternal and Child Health Clinic in downtown Amman, Jordan, which served a mainly lower middle-class urban refugee population. A group of 107 anaemic mothers [Hb <11 g/dl, the international definition of anaemia in pregnancy (18, 19)] was selected at 37 weeks' gestation to avoid pre-term births, and matched as closely as possible for age and parity with a control group of 125 non-anaemic mothers (for detailed methodology see Ref. 17). A borderline group of 17 mothers with Hb 11–11.2 g/dl was analysed with controls. The two groups were of similar socio-economic background, as indicated by income, husband's occupation, housing (owned or rented), mother's education, with the main difference between the groups being maternal anaemia, related to fewer iron prescriptions antenatally. The sample was socially representative of the urban lower middle-class in Jordan.

Birth details were recorded (birth weight, gestation, sex of infant and condition at birth) and 59 cord samples obtained. Infants were reviewed at the clinic at 2 wk and 3, 6, 9 and 12 months to assess growth, current nutrition and recent infection history, and a venous blood sample was taken from 3 months onwards. No significant difference was noted between the two groups of infants in birth weight, gender and gestation, nor in relation to "environmental" factors affecting iron status: feeding methods, infection rates and growth velocity (17). Laboratory tests were postponed if infants [illegible] distortion of results. Infants completing the year of follow-up were 89 born to anaemic mothers and 106 controls. Defaulters were mainly families migrating to Palestine or elsewhere, although 6 infants died in the perinatal period and 9 were withdrawn through illness or haemoglobinopathy.

Laboratory methods

Infant haematology included a full blood count, measured by Coulter Cell Counter (Coulter T-1660, Luton, UK), plasma ferritin, measured by enzyme-linked fluorescent assay (ELFA technique) using the VIDAS system (Bio-Mérieux, France), and, from 6 months of age, for zinc protoporphyrin (ZPP), using an Aviv haematofluorometer (Aviv Biomedical Inc., USA). All tests were performed at Zahran Medical Laboratory, Amman, apart from the ZPP. This was measured at Bradford Royal Infirmary, UK, samples being freighted monthly. Stringent local and international quality control measures were followed.

The criteria used to define iron-deficiency anaemia in the infants were Hb <11 g/dl, the international definition of anaemia in infancy, advocated by the World Health Organisation (WHO) (18, 19) and recognised from 6 months of age, and either plasma ferritin <12 μg/l or ZPP >35 μg/dl whole blood, modelled on a similar study by Colomer *et al.* in Valencia, Spain (13). Incidence represented development of iron-deficiency anaemia at any stage of the year. The definition of anaemia as Hb <11 g/dl at 3 months is inappropriate, as in early infancy a physiological anaemia occurs to stimulate erythropoiesis. Infants with mean corpuscular volume (MCV) <75 fl were screened at 1 yr for haemoglobinopathy, by haemoglobin electrophoresis (cellulose acetate alkaline electrophoresis, confirmed by a column chromatography method specific for HbA2), and 4 infants were subsequently excluded.

Statistical methods

Analyses were performed using SPSS for Windows 6.0 statistical package. Statistical tests included: contingency tables and Mantel-Haenszel chi-square for linear association, bivariate correlation, using the Pearson correlation coefficient, Student's *t*-test of independent groups and a paired samples *t*-test with two-tailed significance and a multiple forward step-wise logistic regression analysis. Anthropometric data were analysed on EPI 6 "Anthro" package, EPI INFO (17). Statistical significance was accepted as $p = <0.05$.

Table 1. Mean haemoglobin of subject and control infants in cord blood samples at birth, and at 3–4, 6, 9 and 12 months of age[a]

	Cord	3–4 months	6 months	9 months	12 months
Reference range (18–20)	(14–20 g/dl)		(11–14.5 g/dl)	(11–14.5 g/dl)	(11–14.5 g/dl)
n	22	85	89	79	89
Subject group infants	15.2	10.4	10.6	10.3	10.2
(SD ±)	(1.5)	(0.6)	(0.9)	(0.5)	(0.9)
n	26	106	106	100	106
Control group infants	15.2	10.7	10.8	10.8	10.5
(SD ±)	(1.5)	(0.8)	(0.8)	(0.9)	(0.9)
p-Value	0.9	0.1	0.07	0.001	0.02

[a] t-Test for means of independent groups, 2-tailed significance, SPSS.

Results

The mean haemoglobin was lower than the reference values both in cord blood samples and at 3, 6, 9 and 12 months of age (18–20) (Table 1). There was a high incidence of anaemia throughout the year (Table 2). The overall incidence of iron-deficiency anaemia during the first year of life was 72% with significantly higher incidence of 81% in infants born to anaemic mothers ($n=91$) compared to 65% in those born to non-anaemic controls ($n=112$) ($p=0.01$) (17). This does not strictly represent prevalence, as not all infants could be tested at each visit, but once infants became iron-deficient, they remained so throughout the year despite iron supplementation. Iron deficiency was associated with lower infant birthweight, (4.4% <2500 g; 19% <3000 g) and "catch-up" growth (17). Table 3 shows the number of infants tested and the percentage with iron-deficiency anaemia at 6 and at 12 months of age. Table 4 shows mean values of iron status indicators in the two groups of infants at 12 months of age.

Some infants had low MCV and mean cell haemoglobin (MCH) with anaemia while ferritin and ZPP were within normal limits, even after exclusion of haemoglobinopathy. Conversely, in some "cases" of iron-deficiency anaemia with Hb <11 g/dl and plasma ferritin <12 μg/l, MCV and MCH were within the normal range: 15% MCV >73 fl and 19% MCH >23 pg. Approximately 10% of the infants were anaemic with a haematological profile inconsistent with iron deficiency: these were excluded from the analysis. Table 5 highlights the problem involved in defining iron-deficiency anaemia. The results represent the whole sample of infants and are cross-sectional at 6 and at 12 months of age. The incidence of anaemia is shown, and the percentage of infants identified as iron-deficient also, using various haematological indicators. The criteria of either plasma ferritin <12 μg/l or ZPP >35 μg/dl whole blood identified more "cases" of iron-deficiency anaemia than one indicator alone or both together. ZPP identified marginally more "cases" than plasma ferritin.

Discussion

The definition of anaemia, and the criteria for iron-deficiency anaemia have been used in an arbitrary manner despite international guidelines (18, 19), making comparison of findings difficult. The high incidence of anaemia found in the Jordanian infants raises the question as to whether appropriate haematological indices were used. A large UK study (14) reported that 23% of infants were anaemic at 8 months of age ($n=1075$), but only 1.2% had plasma ferritin <12 μg/l ($n=754$). The authors challenged the international definition of anaemia in infancy in the UK setting, suggesting a lower cut-off value for haemoglobin. In the light of the risk to infant development reported with Hb <11 g/dl (3), the WHO criterion would seem appropriate. Our results are comparable with other prevalence data from Jordan of 62–75% anaemia in infancy (6, 8–12). In contrast to our study, Colomer *et al.* (13), in a middle-class setting

Table 2. The percentage of infants in subject and control groups with haemoglobin below various "cut-off" criteria, at 6, 9 and 12 months of age

	6 months		9 months		12 months	
Hb	Subject ($n=89$)	Control ($n=106$)	Subject ($n=79$)	Control ($n=100$)	Subject ($n=89$)	Control ($n=106$)
<11 g/dl(%)	68	58	77	58	79	66
<10.5 g/dl(%)	39	34	56	33	54	45
<10 g/dl(%)	20	14	34	18	35	26

Table 3. The incidence of iron-deficiency anaemia in the two groups at 6 and at 12 months of age[a]

	6 months		12 months	
	Subject ($n=89$)	Control ($n=106$)	Subject ($n=89$)	Control ($n=106$)
% IDA	41	29[b]	65	50[b]

[a] According to research criteria of Hb <11 g/dl and either plasma ferritin <12 μg/l or zinc protoporphyrin >35 μg/dl whole blood (18, 20).
[b] $p = <0.05$, chi-square for linear association, SPSS.

in Spain, with normal birthweight infants, found only 9% iron-deficiency anaemia in infants who completed the 1 yr follow-up ($n=113$). This may reflect the different socio-economic settings: iron-deficiency is more prevalent in developing countries, and possibly feeding practices are different. A similar low prevalence was reported in the USA (15) and in the UK (2). While infants in Jordan are often breastfed for 12 months, weaning foods deficient in iron were introduced early in our study (17), vastly diminishing bioavailability of breast milk iron (21). A therapeutic response to iron supplementation was a prerequisite for diagnosis in the Spanish study (13). However, routine referral of anaemic infants for treatment in our study did not improve the anaemia, as shown by subsequent testing.

Yip *et al.* (6, 7) interpreted the high prevalence of anaemia in mothers and infants in the UNWRA survey as indicative of iron deficiency, since women of child-bearing age and infants are vulnerable to iron deficiency. No further indicators were considered necessary in the screening programme. Dallman *et al.* (22) suggest that one single abnormal iron status indicator, e.g. a low haemoglobin or haematocrit, is sufficient to warrant a therapeutic trial of iron. If more than one indicator is used, treatment should be on the basis of "either/or" rather than both indicators being abnormal. Our results show that classification of iron deficiency in anaemic infants, using plasma ferritin and zinc protoporphyrin with an "either/or" approach (22) identified categorically many cases of iron-deficiency anaemia. Substantially more were identified than if only one indicator was used, or if both were required to meet classification criteria (Table 5).

In a survey of school children in Zanzibar (23), haemoglobin, plasma ferritin and ZPP "showed a strong relationship in the expected direction". A similar strong relationship was found in our study, but only in 29% of the infants. Using similar criteria to the USA survey (15), only 29% of the Jordanian infants would have been identified as having iron-deficiency anaemia, although 83% were anaemic between 6 and 12 months, and 72% were diagnosed as iron-deficient by one abnormal indicator in addition to a low haemoglobin.

Results of different iron status indicators were not always consistent with the overall haematological picture. Several indicators are influenced by infection (1, 12, 24–27). Both plasma ferritin and ZPP may become elevated (24), while haemoglobin and transferrin saturation are depressed in the presence of infection (25). In poor communities with frequent, even sub-clinical infant infections, laboratory results may be obscured for several weeks after recovery, especially a raised plasma ferritin. A study in South Africa showed that serum ferritin related well to anaemia in elite Johannesburg pre-school children, but had no relation to anaemia in the poor children of Soweto, although geographically relatively close (27). Madanat *et al.* reported that only 30% of iron-deficient Jordanian children had plasma ferritin <12 μg/l (12). Transferrin receptor is considered by some authors to be more reliable

Table 4. Mean values of iron status indicators in subject and control infants at 12 months of age[a]

Reference range (13, 18, 19) n	HB (11–14.5 g/dl) 89	HT (33–44%) 89	RBC (3.9–5.2 × 10.12/1) 89	MCV (74–84 fl) 89	MCH (24–28 pg) 89	MCHC (310–350 g/l) 89	Plasma ferritin (12–300 μg/l) 87	ZPP (35 or less μg/dl whole blood) 83
Subject group infants (SD ±) n	10.2 (0.9) 106	32 (2.2) 106	4.7 (0.4) 106	69 (5.6) 106	22 (2.5) 106	314 (15.3) 106	13 (13) 104	44 (28) 100
Control group infants (SD ±) p-Value	10.5 (0.9) 0.02	32.5 (2.6) 0.2	4.6 (0.4) 0.5	70 (5.1) 0.06	23 (2.1) 0.008	319 (10.0) 0.006	17 (16) 0.09	40 (21) 0.3

[a] t-Test for means of independent groups, 2-tailed significance, SPSS.

Table 5. The number of infants in the whole study sample identified as anaemic (Hb < 11 g/dl), and the number identified as iron-deficient also, using various haematological parameters at 6 and 12 months of age

	At 6 months ($n=195$)	At 12 months ($n=195$)
Anaemic infants, (Hb < 11 g/dl)	62%	72%
Anaemic infants identified as iron-deficient, using either plasma ferritin < 12 µg/l or ZPP > 35 µg/dl	34%	57%
Infants identified if only plasma ferritin < 12 µg/l was used.	16%	37%
Infants identified if only ZPP > 35 µg/dl. was used	25%	40%
Infants identified if both ferritin and ZPP parameters applied	7%	29%
Infants identified on the basis of Hb < 11 g/dl and either abnormal values of ferritin or ZPP, or both MCV < 72 fl and MCH < 24 pg	41%	65%

(28). ZPP was found to be a useful indicator (29), and a useful alternative in our study.

The results of this study exemplify "the problem of the definition of anaemia" (16), while attempting to evaluate the indicators used. Our results support the use of an "either/or" approach for identifying iron deficiency (22). Alternatively, where iron deficiency is prevalent in a population as in infants in developing countries, a haemoglobin below 11 g/dl may be considered as diagnostic and grounds for closely monitored iron supplementation. A response in a rise of haemoglobin would confirm the diagnosis. With the high prevalence of iron deficiency in infants in developing countries, where iron-fortified weaning foods are often unaffordable, iron supplementation programmes from 6 months or earlier may be the only preventive measure.

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