

# Anaemia during pregnancy as a risk factor for iron-deficiency anaemia in infancy: a case-control study in Jordan

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<b>Background</b>	A high prevalence of 50–65% iron-deficiency anaemia in mothers and infants in Jordan was reported by the United Nations Relief and Works Agency (UNRWA) in 1990. Iron-deficiency in infancy has been shown to delay cognitive and psychomotor development with long-term consequences. While socioeconomic deprivation and inadequate nutrition are known underlying factors, it is unclear whether iron endowment at birth is compromised when mothers are anaemic, further jeopardizing iron status during infancy. A prospective case-control study of infants from birth to one year was conducted in a lower middle-class urban setting in Amman, Jordan. The study objective was to examine the relationship between maternal anaemia and iron-deficiency anaemia during infancy.
<b>Method</b>	A sample of 107 anaemic (Hb <11 g/dl) and 125 non-anaemic mothers was selected at 37 weeks' gestation and matched for age and parity, and infant data at birth obtained. The infants were reviewed at 3, 6, 9 and 12 months, to assess growth, current nutrition, infection rates and iron status. The main outcome measure was the incidence of iron-deficiency anaemia in the two groups of infants, defined in the study as Hb <11 g/dl and either plasma ferritin <12 mcg/l or zinc protoporphyrin >35 mcg/dl.
<b>Results</b>	Iron endowment in cord blood samples appeared similar between the two groups. The incidence of iron-deficiency anaemia was very high in these infants, at 72% by research criteria, (51% if Hb <10.5 g/dl), but significantly higher in the infants born to anaemic mothers at all stages of the year, with overall incidence of 81% (n = 91), compared to 65% in controls (n = 112). This was not explained by differences in environmental risk factors. Anaemic mothers had not recovered adequate iron status at 6 months' postpartum, with implications for future pregnancy iron demands.
<b>Conclusions</b>	Anaemia during pregnancy compromises the health of mothers in traditional cultures, where women tend to have several children close together after marriage, with an inadequate interval to replenish nutritional stores. Their infants also appear to be at increased risk of developing iron-deficiency anaemia, undetected at birth.
<b>Keywords</b>	Anaemia, pregnancy, iron-deficiency, infancy, cord blood
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In 1990 the United Nations Relief and Works Agency for Palestinian Refugees in the Near East (UNRWA) reported maternal and infant iron-deficiency anaemia in 50–65% of the Palestinian population in Jordan, for whom they provide health care.<sup>1</sup> In 1992, the Jordanian Ministry of Health reported maternal anaemia (Hb <11 g/dl)<sup>2,3</sup> in 25% of pregnant women attending government-provided Maternal and Child Health clinics throughout Jordan.<sup>4</sup> No statistics were available for

infants. Iron-deficiency anaemia remains the most common nutritional deficiency worldwide, and is a common feature of infancy both in developed and in developing countries.<sup>5,6</sup> It may lower resistance to infection,<sup>7</sup> and has been shown to delay cognitive and psychomotor development at this critical age,<sup>8–11</sup> which may be irreversible, even after treatment of the anaemia.<sup>9–11</sup> Adverse effects were reported when infant haemoglobin was below Hb 11 g/dl, and increased as anaemia became more severe or chronic.<sup>9,10</sup>

At a time when iron demands for growth are high, infant iron status is often jeopardized by inadequate dietary iron intake and incorrect feeding practices, compounded particularly in developing countries by frequent infections, which compromise appetite and general nutrition.<sup>12</sup>

Several authors suggest a relationship with maternal iron-deficiency anaemia, attributed to reduced fetal iron stores, the endowment of iron at birth to supply needs during early milk feeding.<sup>13–19</sup> This contrasts with the thought that fetal iron storage is independent of maternal iron status, a view largely based on cross-sectional studies which have either found similar cord values for infants born to anaemic and non-anaemic mothers or no significant correlation between maternal and cord iron status indicators, haemoglobin and plasma ferritin.<sup>20–24</sup>

The present research was modelled on a study by Colomer *et al.* in Valencia, Spain,<sup>13</sup> and tested the hypothesis that maternal anaemia is an important risk factor for iron-deficiency anaemia in infants.

While recognizing the effect of haemodilution in lowering haemoglobin levels in late pregnancy, this study was conducted in a community where women have several children in close succession, often with inadequate replenishment of iron stores between pregnancies. It therefore adopted the international criterion of Hb <11 g/dl for anaemia in pregnancy advocated by the World Health Organization (WHO).<sup>2,3</sup> While no reference range exists for haemoglobin in pregnancy in Jordan, Zahran Laboratory, Amman, which was responsible for laboratory tests in this study, also used a lower cutoff of Hb 11 g/dl, based on clinical findings over years. Different criteria have been used by authors for the diagnosis of iron-deficiency anaemia in infancy and the international lower cutoff of Hb 11 g/dl for anaemia in infancy advocated by WHO<sup>2,3</sup> has been challenged in some settings.<sup>25</sup> This study adopted the WHO definition, based on the research findings of a risk to psychological development for infants with Hb <11 g/dl.<sup>9,10</sup>

## Method

A prospective case-control study of infants from birth to one year was conducted (October 1993–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 minimal sample of 97 in each group was selected, to allow detection of a 20% increase in incidence, which may be attributed to reduced fetal iron stores in subject infants ( $P = 0.05$ , power = 80%)

The subject group of 107 anaemic mothers with Hb <11 g/dl 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. Selection was on the basis of a routine clinic capillary haematocrit of Ht <33%

for anaemic mothers or Ht >35% for controls. The research was explained and mothers' verbal consent to participate obtained. A venous sample was taken and mothers were allocated to the subject group if this confirmed anaemia (Hb <11 g/dl). A small borderline group of 17 mothers had Hb 11–11.2 g/dl and were analysed with controls. Anaemic mothers were 25% of women booking at the clinic.<sup>4</sup> The two groups were of similar socio-economic background, with the main difference between the groups being maternal anaemia, related to fewer iron prescriptions antenatally. Mothers were routinely prescribed iron and folic acid antenatally, but there was little post-natal follow-up of iron status. No other dietary information was collected for the mothers.

The maternal profile included: (a) full blood count, measured by Coulter Cell Counter (Coulter T-1660, Luton, UK), (b) plasma ferritin, measured by enzyme-linked fluorescent assay (ELFA technique), using the VIDAS system (Bio-Mérieux, France), (c) serum iron and total iron-binding capacity (TIBC), (Ferrimat-Kit, Bio-Mérieux, France and Photometer 4010, Boehringer, Germany), from which transferrin saturation was calculated, (d) serum B12 and serum folate with Dual Count SPNB (solid phase no boil) technique and radioimmuno-assay (Diagnostic Products Corporation, USA), using labelled cobalt to assess B12 and labelled iodine to assess folate and the LKB Wallac 1270–004 Rackgamma 11 counter (USA).

Birth details of all infants were recorded (birthweight, gestation, sex of infant and condition at birth) and 59 cord samples were obtained. Infants were reviewed at the clinic at 2 weeks to establish a rapport with mothers, to check birth details and feeding method and to commence anthropometric measurements; weight, length and head circumference. Data were plotted on a National Center for Health Statistics growth chart (NCHS, Rockville, Minnesota, USA). Subsequent review was at 3, 6, 9 and 12 months for growth measurements, details of current nutrition and recent infection history obtained through interview; a venous blood sample was taken from 3 months onwards. Laboratory tests were conducted for a full blood count, plasma ferritin and, from 6 months of age, for zinc protoporphyrin (ZPP), using the 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.

Iron-deficiency anaemia was classified in the infants as Hb <11 g/dl, the international definition of anaemia in infancy,<sup>2,3</sup> recognized from 6 months of age, and either plasma ferritin <12 mcg/l or ZPP >35 mcg/dl whole blood.<sup>3,13</sup> The definition of anaemia as Hb <11 g/dl at 3 months is inappropriate, as in early infancy a physiological anaemia occurs to stimulate renewed erythropoiesis. Only those infants with plasma ferritin <12 mcg/l in addition were classified as iron-deficient at this stage. The classification of overall iron status in comparison of the two groups represents development of iron-deficiency anaemia at any stage of the year. In almost all cases, once infants developed iron-deficiency, this persisted throughout the year. Infants were referred for medical attention and 42 were prescribed iron supplementation before 12 months of age, but compliance was poor and only 13 showed a rise in haemoglobin of 0.5–1 g/dl at the subsequent visit. These infants were counted

**Table 1** Maternal haematology: mean values in subject and control mothers<sup>a</sup>

	Antenatal						Postnatal	
	First trimester	37 weeks' gestation					6 months' postnatal	
	Booking Ht <sup>b</sup>	Hb <sup>c</sup>	Plasma ferritin	Transferrin saturation	Serum folate	Serum B <sub>12</sub>	Hb	Plasma ferritin
<b>Reference range<sup>2,3</sup> and Zahran Lab., Amman</b>	33–47%	11–14 g/dl	12–300 mcg/l	>15%	3–17 ng/ml	200–1000 pg/ml	12–16 g/dl	12–300 mcg/l
n	96	107	107	102	103	103	90	90
<b>Anaemic mothers</b>								
Subject group	33.7	9.9	7.6	15	8.2	186.1	11.8	17.4
(SD ±)	(2.9)	(0.7)	(5.5)	(8)	(4.6)	(205.7)	(1.1)	(15.9)
n	113	125	122	117	120	121	107	109
<b>Control group</b>	35.7	12.2	21.9	21.8	10.7	196.8	12.9	39.8
(SD ±)	(3.2)	(0.9)	(21.4)	(8.2)	(5.8)	(155.7)	(0.8)	(36.7)
<b>P-value</b>	***	***	***	***	0.001	0.6	***	***

<sup>a</sup> t-test for means of independent groups, 2-tailed significance, SPSS.

<sup>b</sup> Haematocrit.

<sup>c</sup> Haemoglobin.

\*\*\**P* = <0.0005.

as cases of iron-deficiency anaemia during the first year of life in the analysis, following Colomer *et al.*<sup>13</sup> and haematological data were included in subsequent analysis. Infants with mean corpuscular volume (MCV) <75 fl were screened at one year for haemoglobinopathy, by haemoglobin electrophoresis (cellulose-acetate alkaline electrophoresis, confirmed by column chromatography method specific for HbA2) and four infants were subsequently excluded from the analysis. Maternal iron status was reviewed at 6 months' postpartum to assess postnatal recovery: full blood count and plasma ferritin, and serum B12 if values were low in pregnancy.

Analyses were performed using SPSS for Windows 6.0 statistical package, and anthropometric data were analysed on EPI 6 'Anthro' package, EPI INFO.

Ethical consent was obtained from The Ministry of Health, Jordan, in accordance with guidelines of the WHO.

## Results

### Mothers

There was no significant difference in social background of the two groups as measured by socioeconomic indicators, husband's occupation, income, housing, (owned or rented), years of education, and place of delivery. Home birth, often related to the poorer section of society in developing countries, was 9% for anaemic mothers compared to 6% for controls, and those who delivered in the government hospital for a modest fee were 60% of subjects compared to 58% controls (NS). The whole sample was socially representative of the urban lower middle-class in Jordan.

Table 1 shows maternal haematology of the two groups: in the first trimester, at 37 weeks' gestation and at 6 months' postpartum. Anaemic mothers had significantly lower mean values for all iron status indicators, and serum folate antenatally, which persisted at 6 months' postpartum with mean (standard deviation) Hb 11.8 (1.1) g/dl and plasma ferritin 17.4 (15.9)

mcg/l. Of 200 mothers tested at 6 months' post-partum, 38 had Hb <12 g/dl and 70 had ferritin <12 mcg/l, the international lower reference for iron status of non-pregnant women,<sup>3</sup> with implications for the iron demands of a future pregnancy: 26% of mothers had less than one year inter-pregnancy spacing. A significantly lower clinic haematocrit in early pregnancy in the subject group (Table 1), persisted throughout pregnancy, with or without iron supplementation, emphasizing the importance of a satisfactory iron status at the onset of pregnancy. Anaemic mothers also had significantly fewer prescriptions of haematinics antenatally (*P* = <0.05). Iron supplements were prescribed to 35% of mothers postnatally. Serum B12 was low (<200 pg/ml) in 67% of mothers antenatally, distributed evenly between the groups, and possibly related to haemodilution in pregnancy. However, those mothers with low values were checked at 6 and 12 months, when 34 out of 127, and 19 out of 31 mothers, respectively, had persistently low values.

### Infants

All infants were born after 37 weeks' gestation by study design, to avoid complications of prematurity. Infants studied comprised 124 males and 108 females, evenly distributed between the groups. Mean (SD) birthweight was similar between the two groups: 3308 (515) g for infants born to anaemic mothers (*n* = 107), and 3258 (487) g for controls (*n* = 125) (NS).

There was no correlation between low maternal haemoglobin or plasma ferritin and birthweight. Overall, 4.4% of infants were low birthweight (<2500 g, WHO criterion), small for gestational age infants, indicating nutritional deprivation *in utero*, and 19% weighed <3000 g. Iron-deficiency in infants was associated with lower infant birthweight (*P* = <0.05) and with increased growth velocity of infants and 'catch-up' growth, especially in male infants at 6 months (*P* = <0.01). Mean weight-for-age (WAZ) Z score for the whole group of infants at 12 months of age was -0.65, which corresponds well with the WHO Working Group on Infant Growth<sup>26</sup> report related to the growth pattern

**Table 2** Cord haematology: mean values in subject and control infants<sup>a</sup>

	Cord Hb <sup>b</sup>	Ht <sup>c</sup>	RBC <sup>d</sup>	MCV <sup>e</sup>	MCH <sup>f</sup>	MCHC <sup>g</sup>	Plasma ferritin
<b>Reference range<sup>27</sup> and Zahran Lab., Amman</b>	14–20 g/dl	42–64%	4.2–5.8 × 10 <sup>12</sup> /l	100–110 fl	36–40 pg	340–380 g/l	12–300 mcg/l
n	22	22	22	22	22	22	27
<b>Subject group infants</b>	15.2	46.8	4.4	105.8	34.2	322	195
(SD ±)	(1.5)	(4.7)	(0.4)	(5.9)	(1.9)	(15.2)	(149)
n	26	26	26	26	26	26	32
<b>Control group infants</b>	15.2	46.1	4.4	106.1	34.6	327	200
(SD ±)	(1.5)	(4.4)	(0.4)	(3.9)	(1.6)	(9.7)	(124)
<b>P-value</b>	0.9	0.6	0.5	0.8	0.4	0.2	0.9

<sup>a</sup> t-test for means of independent groups, 2-tailed significance, SPSS.

<sup>b</sup> Haemoglobin.

<sup>c</sup> Haematocrit.

<sup>d</sup> Red blood cells.

<sup>e</sup> Mean corpuscular volume.

<sup>f</sup> Mean corpuscular haemoglobin.

<sup>g</sup> Mean corpuscular haemoglobin concentration.

**Table 3** Infant haematology at 9 months of age<sup>a</sup>

	Hb <sup>b</sup>	Ht <sup>c</sup>	RBC <sup>d</sup>	MCV <sup>e</sup>	MCH <sup>f</sup>	MCHC <sup>g</sup>	Plasma ferritin	ZPP <sup>h</sup>
<b>Reference range<sup>2,3</sup> and Zahran Lab., Amman</b>	11–14.5 g/dl	33–44%	3.9–5.2 × 10 <sup>12</sup> /l	74–84 fl	24–28 pg	310–350 g/l	12–300 mcg/l	≤35 mcg/dl whole blood
n	79	80	87	78	78	78	79	70
<b>Subject group infants</b>	10.3	32	4.6	70	22	318	21	42
(SD ±)	(0.9)	(2.4)	(0.5)	(5.1)	(2.1)	(10.5)	(23)	(22)
n	100	100	87	100	100	100	102	86
<b>Control group infants</b>	10.8	33	4.6	72	23	322	20	37
(SD ±)	(0.9)	(2.2)	(0.4)	(4.8)	(1.9)	(9.2)	(26)	(17)
<b>P-value</b>	0.001	0.006	0.9	0.03	0.01	0.01	0.7	0.1

<sup>a</sup> t-test for means of independent groups, 2-tailed significance, SPSS.

<sup>b</sup> Haemoglobin.

<sup>c</sup> Haematocrit.

<sup>d</sup> Red blood cells.

<sup>e</sup> Mean corpuscular volume.

<sup>f</sup> Mean corpuscular haemoglobin.

<sup>g</sup> Mean corpuscular haemoglobin concentration.

<sup>h</sup> Zinc protoporphyrin.

of breastfed infants. The incidence of growth faltering at 12 months was 17.2%, (defined as  $-2SD$ , when subtracting WAZ at birth from WAZ at 12 months) but this was not associated with iron deficiency. There was no significant difference in growth velocity between the two groups of infants.

Infant haemoglobin values were lower than reference values,<sup>27</sup> both at birth in cord blood samples (mean [SD] Hb 15.2 [0.7] g/dl) ( $n = 59$ ), and throughout the year (Tables 2 and 3). It was only possible to obtain 59 cord samples from the hospitals. Cord haemoglobin ranged from 12.3 to 17.9 g/dl with seven samples below the lower reference level of 14 g/dl.<sup>27</sup> Cord ferritin ranged from 7 to 639 mcg/l, with one sample only  $<12$  mcg/l. An inverse correlation was found between mean cord haemoglobin and plasma ferritin levels, such that those infants with lower cord haemoglobin had higher ferritin, and

those with lower ferritin levels had higher cord haemoglobin ( $P = <0.05$ ) ( $n = 48$ ). Infants with iron sufficiency at 6 months had mean cord Hb 16.4 (1.0) g/dl ( $n = 11$ ) while those with iron deficiency had mean cord Hb 14.7 (1.3) g/dl ( $n = 14$ ) ( $P = <0.01$ ). Mean cord ferritins were 136 (101) mcg/l ( $n = 16$ ), and 196 (140) mcg/l ( $n = 18$ ), respectively (NS) (Cord log. ferritin  $P = 0.06$ ). A significant 'tracking' was noted between values of iron status indicators, haemoglobin and ferritin. Infants with higher values in earlier samples tended to have higher values later in infancy, emphasizing the importance of fetal iron endowment (Table 4).

#### Correlation between the two groups

Mean cord blood values suggested similar iron endowment at birth (Table 2). No correlation was found between maternal and

**Table 4** Correlation between infant iron status indicators throughout the year<sup>a</sup>

Iron status indicator	Age (months)	r	P-value	n
<b>Cord Hb<sup>b</sup>-Hb</b>	3	0.36	<0.05	35
	6	0.51	<0.01	38
<b>Hb</b>	3-6	0.39	<0.0005	152
	3-9	0.33	<0.0005	143
	3-12	0.16	0.05	149
<b>Cord ferritin-ferritin</b>	3	0.39	<0.01	47
	6	0.26	0.08 (NS)	48
<b>Ferritin</b>	3-6	0.47	<0.0005	165
	3-9	0.41	<0.0005	153
	3-12	0.41	<0.0005	156
	6-9	0.36	<0.0005	173
	9-12	0.20	<0.01	168
<b>ZPP<sup>c</sup></b>	6-9	0.58	<0.0005	116
	6-12	0.55	<0.0005	133
	9-12	0.72	<0.0005	139

<sup>a</sup> Bivariate correlation: Pearson correlation coefficient (r) and P-value, 2-tailed significance, SPSS.

<sup>b</sup> Haemoglobin.

<sup>c</sup> Zinc protoporphyrin.

**Table 5** Mean infant haemoglobin (Hb) levels between the two groups,<sup>a</sup> and percentage of infants with Hb below various cutoff criteria at 6, 9 and 12 months

	6 months		9 months		12 months	
	Subject (n = 89)	Control (n = 106)	Subject (n = 79)	Control (n = 100)	Subject (n = 89)	Control (n = 106)
<b>Mean Hb (SD) (g/dl)</b>	10.6 (0.8)	10.8 (0.8)	10.3 (0.9)	10.8 (0.9)**	10.2 (0.9)	10.5 (0.9)*
<b>Median Hb</b>	10.7	10.7	10.3	10.8	10.3	10.6
<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

<sup>a</sup> t-test for means of independent groups, 2-tailed significance, SPSS.

\*P = <0.05; \*\*P = <0.01.

cord haemoglobin and ferritin values. But haemoglobin values were consistently lower for infants born to anaemic mothers throughout the year, reaching significance from 9 months ( $P = <0.005$ ) (Tables 3 and 5). At 9 months of age, 77% of subject infants had Hb <11 g/dl compared with 58% of controls, and 34% of subjects had Hb <10 g/dl compared with 18% of controls.

The incidence of anaemia (Hb <11 g/dl)<sup>2,3</sup> between 6 and 12 months was very high at 83%. Using the research criteria of Hb <11 g/dl and either plasma ferritin <12 mcg/l or ZPP >35 mcg/dl<sup>3,13</sup> the incidence of iron-deficiency anaemia was 72% (51% if Hb <10.5 g/dl was taken as lower reference). It was significantly higher at all stages of the year for infants born to anaemic mothers, with overall incidence of 81% (n = 91), compared with 65% for controls (n = 112) ( $P = 0.01$ ) (Table 6). (At 3-4 months of age 75% of infants had Hb <11 g/dl, which may correspond with physiological anaemia. Six infants also had plasma ferritin <12 mcg/l, and were defined as iron-deficient; confirmed at a subsequent visit.) The presence of iron deficiency was further supported by a predominantly hypochromic, microcytic red cell morphology with anisocytosis. Mean corpuscular

volume, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were found to be significantly lower in the infants born to anaemic mothers from 9 months of age (Table 3). Table 6 shows the number of infants tested and the percentage of infants with iron-deficiency anaemia at 3-4, 6, 9 and 12 months according to research criteria. The number of cases has been shown at each stage of the year and a cumulative incidence in numbers and percentage for each group calculated from this. Most infants remained iron-deficient at the subsequent visit but as not all infants could be tested at every stage, this does not strictly represent prevalence. The difference in number of cases between the groups increased to 20% by 9 months of age, while the gap was reduced to 16% at 12 months. This demonstrates accelerated development of iron-deficiency anaemia of approximately 3 months for infants born to anaemic mothers.

#### Environmental factors

Environmental factors did not explain the difference in incidence of iron deficiency in the two groups of infants. Both were from a similar disadvantaged urban background. Overall

**Table 6** The incidence of iron-deficiency anaemia (IDA) in the two groups at 3–4, 6, 9 and 12 months

	3–4 months		6 months		9 months		12 months	
	Subject (n = 88)	Control (n = 112)	Subject (n = 89)	Control (n = 106)	Subject (n = 79)	Control (n = 100)	Subject (n = 89)	Control (n = 106)
% IDA	6	1*	41	29*	57	43*	65	50*
New cases	5	1	34	30	23	23	12	19
Cumulative no. cases	5	1	39	31	62	54	74	73
Cumulative incidence (%)	6	1	43	28	68	48	81	65*
							(n = 91)	(n = 112)

\* $P = <0.05$ ,  $\chi^2$  for linear association, SPSS.

**Table 7** Characteristics of the most relevant feeding practices between the two groups

	Subject	Control	P-value <sup>a</sup>
Breastfeeding at 12 months of age (%)	60 (n = 95)	59 (n = 117)	0.8
Duration exclusive breastfeeding (× [SD] months)	2.3(1.6) (n = 99)	2.3(1.6) (n = 121)	0.8
Age of introduction of solids (× [SD] months)	3.2(1.2) (n = 97)	3.2(1.2) (n = 121)	0.9
Consumption of cows' milk, including desserts <6 months (%)	26 (n = 97)	34 (n = 119)	0.2
Consumption of tea at 6 months (%)	35 (n = 97)	35 (n = 120)	0.9
Consumption of heme iron at 9 months (once per week or <) (%)	26 (n = 89)	28 (n = 105)	0.4

<sup>a</sup>  $\chi^2$  for linear association, SPSS.

infection rates between subject and control infants were similar: mean 26.7 (27.7) days' respiratory infection and mean 7.8 (5.9) days' diarrhoeal disease respectively, impacting on iron status at 9 months. Of 30 stool samples obtained, two were positive for parasites. Feeding practices were also similar between the groups (Table 7). Infants were almost universally breastfed, (90% at 6 months and 60% at one year), but only 30% were still exclusively breastfed at 3 months. The high bio-availability of breast milk iron is impaired by early introduction of supplementary foods.<sup>28</sup> Herbal drinks were frequently used, 63% of mothers introduced solids before 4 months, mainly yoghurt and cows' milk desserts low in iron, and 35% of infants were taking tea, an inhibitor of iron absorption at 6 months. Infant weaning diet was mainly family food, with little meat or vitamin C in meals to enhance absorption. Frequent infections and 'teething' appeared to depress appetite and promote chronic dependence on breastfeeding and refusal of foods by some infants later in the year.

## Discussion

The adverse effects of iron-deficiency anaemia on cognitive and psychomotor development in the critical rapid growth period of infancy have been well documented.<sup>8–11</sup> Nevertheless, it remains the most prevalent nutritional deficiency worldwide, affecting both developed and particularly developing countries.<sup>5,6</sup> The high prevalence found in these Jordanian infants and reported by UNRWA represents a serious public health problem.

The majority of infants were otherwise healthy and the findings raise the question as to whether appropriate haematological

measures were used. The Avon Longitudinal Study of Pregnancy and Childhood, UK<sup>25</sup> challenged the WHO lower reference criterion for anaemia in infancy of Hb 11 g/dl in the UK setting. Authors reported 23% of infants at 8 months of age with capillary Hb <11 g/dl (n = 1075), while only 1.2% had plasma ferritin <12 mcg/l (n = 754) and 0.4% both low Hb and ferritin. However, in the light of the risk to cognitive and psychomotor development found for infants with Hb <11 g/dl,<sup>9,10</sup> the international reference was adopted in the present study as most appropriate. Table 5 shows a high incidence of anaemia in infants, even with a cutoff of Hb 10.5 or 10 g/dl. Iron-deficiency anaemia was defined as Hb <11 g/dl, confirmed by either plasma ferritin <12 mcg/l or ZPP >35 mcg/dl whole blood, supported by a microcytic hypochromic blood film. Since ferritin is an acute phase reactant, often elevated in infancy with even sub-clinical infections, ZPP was a useful alternative indicator. According to these criteria 72% of infants were affected, 81% born to anaemic mothers (n = 91) and 65% to non-anaemic controls (n = 112) ( $P = 0.01$ ). Colomer *et al.*,<sup>13</sup> in a middle-class setting in Spain using the same criteria, found a 17% incidence of iron-deficiency anaemia during the first year of life in infants born to anaemic mothers (n = 47) compared to 3% for controls (n = 66) (odds ratio = 6.57, 95% CI : 1.81–25.97). Most of those infants were fed iron-fortified formula from birth.

While environmental risk factors affect dietary iron intake and utilization (poor diet, faulty feeding practices, infections or presence of parasites), the amount of iron endowment at birth also affects subsequent iron status. Iron crosses the placenta by active transport, and the fetus accumulates iron stores in the liver, spleen and bone marrow, mainly from 32 weeks'

gestation, as an endowment to meet iron requirements during early milk feeding, when dietary iron intake is low.<sup>27,29</sup> Pre-term and low birthweight infants are at risk of iron deficiency through reduced fetal iron stores<sup>27</sup> but whether iron endowment is adequate when mothers are anaemic has been unclear. The high prevalence of maternal and infant anaemia often found in developing countries may be associated.

The results of this study support the hypothesis that maternal anaemia is an important risk factor for subsequent iron deficiency in the infant, though cord values suggested independence of fetal iron endowment. Mean cord values for haemoglobin and ferritin were similar between the two groups (Table 2), and no correlation was found between maternal and cord haemoglobin or ferritin levels, even when lower and higher cutoff values were compared. A cross-sectional study at birth would conclude that there was no association, but follow-up of infants showed significantly higher incidence of iron-deficiency anaemia in those infants born to anaemic mothers at each 3-monthly stage, with a cumulative incidence of 81% (n = 91) compared to 65% (n = 112) for infants born to non-anaemic mothers (Table 6).

Overall mean cord haemoglobin for the 59 samples obtained was lower than reference values,<sup>27</sup> with mean cord Hb 15.2 (0.7) g/dl. Cord anaemia has been reported in other studies in developing countries, and may be a useful indicator or risk factor for anaemia in infancy.<sup>19</sup> It has been associated with haemoglobinopathy, moderate to severe maternal iron-deficiency anaemia and with malaria in pregnancy.<sup>19</sup> Haemoglobinopathy as a confounder was addressed in this study and Jordan is not a malaria-endemic region. Anaemia in pregnancy in this study was mild-moderate (mean maternal Hb 9.9 [0.7] g/dl) and was not reflected in a lower mean cord blood haemoglobin in subject infants compared to controls (Table 2). Conflicting findings have been reported in cord values in relation to anaemia in pregnancy in developing countries. In studies from India, Ahmad *et al.*<sup>17</sup> reported significantly higher cord haemoglobin levels for infants born to non-anaemic mothers than for those born to anaemic, iron-deficient mothers. Singla *et al.*<sup>18</sup> found a significant association between mean cord and maternal haemoglobin levels at all degrees of maternal iron status from Hb <6 g/dl to iron-sufficient mothers. Mean cord haemoglobin was 15.6 (2.1) g/dl in relation to the lowest mean maternal Hb 8.6 (0.8) g/dl (n = 85) (r = 0.73) (P = <0.001). In contrast, in a study by Bhargava *et al.*<sup>23</sup> there appeared to be a compensatory mechanism to protect infants born to severely anaemic mothers (Hb <6 g/dl), who had significantly higher mean cord haemoglobin and ferritin levels (mean Hb 19.1 (2.4) g/dl and ferritin 101.6 (1.49) ng/ml (n = 12)) than those born to less anaemic mothers. (All cord ferritin levels were low in this study.) This elevation in cord haemoglobin is thought to reflect fetal hypoxia and has been associated with intra-uterine growth retardation.<sup>19</sup> Both Singla and Bhargava reported a low mean birthweight for infants born to anaemic mothers, 2186 (444) g and 2183 (397) g respectively, which is a risk for developing iron-deficiency during infancy through reduced fetal iron stores,<sup>27</sup> as was lower birthweight in the infants in this study, though not related to maternal anaemia.

A significant inverse correlation was found between cord haemoglobin and ferritin for the Jordanian infants, and was also reported by MacPhail *et al.*<sup>30</sup> Those infants with higher

haemoglobin had lower ferritin at birth and those with lower haemoglobin had higher plasma ferritin (n = 48) (P = <0.05). This suggests that lack of correlation between maternal and cord haemoglobin or ferritin does not exclude a relationship between maternal and infant iron status at birth.

The findings agree with those of several prospective studies.<sup>13–15</sup> Colomer *et al.*<sup>13</sup> did not document cord values, but found a significant increase in incidence of iron-deficiency anaemia in infants born to anaemic mothers than to non-anaemic controls. Morton *et al.*,<sup>14</sup> in a UK setting, found similar mean values for cord haemoglobin and ferritin for infants born to anaemic and non-anaemic mothers, as in the present study, but a significant correlation of maternal iron status, with infant iron status at 6 and 12 months. Preziosi *et al.*<sup>15</sup> in a study in Niger reported significantly higher serum ferritin concentrations at 3 months of age in infants born to iron-supplemented mothers than in those born to a placebo-supplemented control, when cord blood values suggested similar endowment of iron between the two groups. In contrast, Murray *et al.*<sup>24</sup> also in Niger, found similar haemoglobin and transferrin saturation in cord samples and at 6 months in infants born to anaemic and non-anaemic mothers. Cord haemoglobin was high: mean Hb 18.9 (0.9) compared to mean Hb 18.7 (1.3) for controls. 'Tracking' of values of iron status indicators detected in the present research also endorses findings of several authors,<sup>14,31</sup> emphasizing the importance of endowment at birth. However, iron status indicators in cord blood are measured at a time of transition from the relatively hypoxic intra-uterine environment, a time of swift physiological change, which may explain conflicting results.<sup>20</sup> While cord values did not predict the significantly increased incidence of subject infants developing iron-deficiency anaemia later in the year, the results of this study suggest that maternal anaemia may be a predictor of increased risk.

Environmental factors must contribute to the very high incidence of anaemia (65% even in the control group). Breastfed infants exhaust iron stores by 6 months, and iron supplementation or fortification of foods is recommended thereafter.<sup>32</sup> But no significant difference was detected between the groups in socioeconomic status, feeding practices or infection rates to account for the increased prevalence in subject infants. Further research into breast milk iron content and availability when mothers are anaemic would be valuable.

While these findings have relevance especially for developing countries, a higher prevalence of iron deficiency has also been reported for infants in ethnic minorities in the UK.<sup>2,5</sup> Anaemia in pregnancy compromises the health of mothers who, as in Jordan, traditionally have several children close together soon after marriage, and usually breastfeed for at least one year in a cycle of pregnancy and lactation, with an inadequate interval to replenish nutritional stores. Their infants also appear to be at increased risk of developing iron-deficiency anaemia, which may be undetected in cord blood values. Although iron absorption in late pregnancy is vastly enhanced, this presupposes adequate iron in the diet, which is not guaranteed in poorer societies, lending support to the argument for routine supplementation in this context. Health initiatives to prevent maternal anaemia and promote full recovery postpartum, including replenishment of storage iron, and to stress the importance of birth-spacing for mother and offspring, would alleviate this potential risk factor for iron deficiency in infancy.

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