

IRON STATUS, SERUM FOLATE AND B₁₂ VALUES IN PREGNANCY AND POSTPARTUM: REPORT FROM A STUDY IN JORDAN

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Background: A high prevalence of anemia has been reported in Jordan affecting women of childbearing age and infants/preschool children. This paper considers maternal iron, folate and B₁₂ status, with possible implications for both maternal and infant health.

Patients and Methods: A case-control study of infants born to anemic (Hb <11 g/dL) (n=107) and non-anemic mothers (n=125) from birth to one year, was conducted in a lower middle-class urban setting in Amman, Jordan. Maternal hematology included full blood count (FBC), plasma ferritin, transferrin saturation, serum folate and B₁₂ at term, and FBC and ferritin at 6 months postpartum. Serum B₁₂ was reassessed at 6 and 12 months postpartum if antenatal values were low. Infant FBC and ferritin were assessed in cord blood and at 3, 6, 9 and 12 months, and zinc protoporphyrin (ZPP) from 6 months.

Results: Anemic mothers (mean [SD] Hb 9.9 [0.7] g/dL) had significantly lower antenatal values for Hb, MCV, MCH, transferrin saturation, plasma ferritin and serum folate than non-anemic mothers (mean Hb 12.2 [0.9] g/dL), which persisted at 6 months postpartum. Antenatal B₁₂ values were low (<200 pg/mL) in 67% of samples (26% <100 pg/mL), evenly distributed between the groups, and, therefore, not related to maternal anemia. Low values persisted in 27% (n=127) and in 61% (n=31), respectively, at 6 and 12 months postpartum. Iron-deficiency anemia (Hb <11 g/dL and either ferritin <12 mcg/L or ZPP >35 mcg/dL) affected 72% of infants, with significantly higher incidence in those born to anemic mothers. Ambiguous hematology in 11% of infants may have reflected other nutritional deficiencies, including vitamin B₁₂, where mothers had depleted values.

Conclusion: Iron, folate and B₁₂ status should be monitored during pregnancy/lactation in developing countries, where nutritional deprivation is more prevalent and women of childbearing age often have a high fertility rate and inadequate interpregnancy interval to replenish body stores. Infant health may also be at risk through a compromised endowment of these micronutrients at birth.

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In 1990, the United Nations Relief and Works Agency for Palestinian Refugees in the Near East (UNRWA) reported a high prevalence of anemia in the Palestinian population in Jordan, for whom they provide health care.¹ Based on international criteria,^{2,3} anemia affected 23% of non-pregnant women of child-bearing age (n=351) and 53.7% of women in the last trimester of pregnancy (n=404). The highest prevalence of 65% was in infants 6-12 months of age (n=406).¹ The 1992 Jordanian Ministry of Health statistics for anemia in pregnancy⁵ and a 1996 national survey of women of childbearing age⁶ were similarly high and reported low ferritin values, indicating iron deficiency. No statistics were

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Accepted for publication 29 July 2000. Received 29 September 1999. available on maternal serum folate or B₁₂ values.

Most of the anemia in women of childbearing age and during pregnancy is related to iron deficiency.^{4,7} However, both low serum folate or B₁₂ have recently been reported in populations in developing countries, with particular respect to pregnant and lactating mothers and their infants.⁸⁻¹³ There may be a higher incidence of folate and vitamin B₁₂ deficiency in the populations of developing countries than previously suspected.

It is thought that iron deficiency in infancy has long-term serious consequences for cognitive and psychomotor development.¹⁴⁻¹⁶ Evidence of developmental delay has been associated more severely, though more rarely, with vitamin B₁₂ deficiency,^{11,17} manifested as a failure to thrive and mental retardation if not corrected.

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The aim of this study was to explore the relationship between anemia in pregnancy and the development of iron-deficiency anemia in infancy.¹⁸ Results in this paper address

maternal iron, folate and B₁₂ status antenatally and up to 12 months postpartum, with discussion of possible implications for both maternal and infant health.

TABLE 1. Maternal hematology: mean values in subject and control mothers, antenatally and at 6 and 12 months postpartum.^{†,‡}

	Term		6 months postpartum		12 months postpartum	
	Subject	Control	Subject	Control	Subject	Control
Mean Ht% (1 st trimester) (ref. 33-37 ^{2,3})	33.7 (2.9) (n=96)	35.2 (3.2)** (n=113)				
Mean Hb (SD) (g/dL) (11-14 in pregnancy) (12-16 postpartum)	9.9 (0.7) (n=107)	12.2 (0.9)** (n=125)	11.8 (1.1) (n=90)	12.9 (0.8)** (n=107)		
Mean MCV (fl) (81-99)	79.4 (8.1) (n=107)	87.8 (5.6)** (n=124)	79.6 (7.2) (n=90)	85 (4.5)** (n=107)		
Mean MCH (pg) (27-31)	25.4 (1.9) (n=107)	29.1 (2.2)** (n=124)	25.7 (2.9) (n=90)	28 (1.8)** (n=107)		
Mean RBC (x10 ¹² /L) (4.2-5.5)	3.9 (0.4) (n=107)	4.2 (0.4)** (n=124)	4.6 (0.4) (n=90)	4.6 (0.3) (n=107)		
Mean transferrin saturation (%) (>15)	15 (8) (n=102)	22 (8)** (n=117)				
Plasma ferritin (mcg/L) (12-300)	7.6 (5.5) (n=107)	21.9 (21.4)** (n=122)	17.4 (15.9) (n=90)	39.8 (36.7)** (n=109)		
Mean serum folate (ng/mL) (3-17)	8.2 (4.6) (n=103)	10.7 (5.8)* (n=120)				
Mean serum B ₁₂ (pg/mL) (200-1000)	186 (206) (n=103)	197 (156) (n=121)	289 (58) (n=58)	275 (130) (n=69)	199 (82) (n=13)	175 (46) (n=18)

*P<0.01; **P<0.0005; †t-test for means of independent groups, 2-tailed significance, SPSS; ‡ some of this data has been published.^{18,31}

TABLE 2. Mean hemoglobin, MCV, MCH, RBC, plasma ferritin and ZPP levels in the two groups of infants[†] and percentage of infants with Hb <11 g/dL and Hb <10.5 g/dL at 6, 9 and 12 months of age.[‡]

	6 months		9 months		12 months	
	Subject (n=89)	Control (n=106)	Subject (n=79)	Control (n=100)	Subject (n=89)	Control (n=106)
Mean Hb (SD) (g/dL) (ref. 11-14.5) ^{2,3,19}	10.6 (0.8)	10.8 (0.8)	10.3 (0.9)	10.8 (0.9)**	10.2 (0.9)	10.5 (0.9)*
<11 g/dL (%)	68	58	77	58	79	66
<10.5 g/dL (%)	39	34	56	33	54	45
Mean MCV (fl) (ref. 74-84)	73 (4.6)	74 (4.6)	70 (5.1)	72 (4.8)*	69 (5.6)	70 (5.1)
Mean MCH (pg) (ref. 24-28)	23.8 (1.9)	24.2 (1.9)	22 (2.1)	23 (1.9)*	21.7 (2.5)	22.6 (2.1)**
Mean RBC (x10 ¹² /L) (ref. 3.9-5.2)	4.4 (0.3)	4.4 (0.4)	4.6 (0.5)	4.6 (0.4)	4.7 (0.4)	4.6 (0.4)
Mean ferritin (mcg/L) (ref. 12-300)	35 (33) (n=70)	42 (41) (n=80)	21 (23) (n=70)	20 (26) (n=86)	13 (13) (n=83)	17 (16) (n=100)
Mean ZPP (mcg/dL) (ref. 35 or <)	39 (21)	39 (12)	42 (22)	37 (17)	44 (28)	40 (21)

*P<0.05; **P<0.01; †t-test for means of independent groups, 2-tailed significance, SPSS; ‡ some of this data has been published.^{18,31}

Method

A prospective case-control study of infants from birth to one year was conducted (October 1993 to June 1996) in a Ministry of Health Maternal and Child Health Clinic in downtown Amman, Jordan, which served a mainly lower middle-class urban refugee population. The subject group of 107 anemic mothers with Hb <11 g/dL² was selected at 37 weeks' gestation to avoid complications of prematurity,

and matched as closely as possible for age and parity with a control group of 125 non-anemic mothers (detailed methodology¹⁸). Twenty-five percent of women booking at the clinic were anemic,⁵ despite routine iron/folic acid antenatal supplements.

The maternal antenatal profile included full blood count (FBC), measured by Coulter Cell Counter (Coulter T-1660, Luton, UK) and plasma ferritin, measured by enzyme-linked fluorescent assay (ELFA technique), using

the VIDAS system (Bio-Mérieux, France). Serum iron and total iron-binding capacity (TIBC) were assessed (Ferrimat-Kit, Bio-Mérieux, France and Photometer 4010, Boehringer, Germany), and transferrin saturation was calculated. Serum B₁₂ and serum folate were measured with the Dual Count SPNB (solid phase no boil) technique and radioimmunoassay (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). Maternal FBC and plasma ferritin were assessed at 6 months postpartum, and serum B₁₂ as well if values had been low in pregnancy, with a further sample at 12 months postpartum if mothers had persistent low serum B₁₂ (<200 pg/mL) at 6 months.

Infant birthweight, gestation, gender and 59 cord blood samples were obtained and infants were reviewed at the clinic at 2 weeks, 3, 6, 9 and 12 months to assess growth, current nutrition and recent infection history.¹⁸ A venous blood sample was taken from 3 months onwards for FBC, plasma ferritin, and from 6 months of age, for zinc protoporphyrin (ZPP), using the Aviv hematofluorometer (Aviv Biomedical Inc., USA). Stringent local and international quality control measures were followed. Iron-deficiency anemia was defined as Hb <11 g/dL and either plasma ferritin <12 mcg/L or ZPP >35 mcg/dL whole blood.^{3,19} Infants with MCV <75 fl were screened at one year for hemoglobinopathy, by hemoglobin electrophoresis (cellulose-acetate alkaline electrophoresis, confirmed by column chromatography method specific for HbA₂) and four infants with hemoglobinopathy were subsequently excluded from the analysis.

Analyses were performed using SPSS for Windows 6.0 statistical package, and anthropometric data were analyzed on EPI 6 "Anthro" package, EPI INFO. Statistical significance was accepted as $P \leq 0.05$.

Results

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. The whole sample was socially representative of an urban lower middle-class group in Jordan.

Anemic mothers, with a mean (SD) Hb 9.9 (0.7) g/dL (n=107) compared with a mean Hb 12.2 (0.9) g/dL (n=125) in controls, had significantly lower mean values for all iron status indicators antenatally. This included "booking" hematocrit in the first trimester, and hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), transferrin saturation and plasma ferritin at term ($P \leq 0.0005$) (Table 1). Anemic mothers also had significantly fewer prescriptions of iron and folic acid antenatally ($P \leq 0.05$). Lower iron status persisted in 54% of the subject mothers (n=90) at 6 months postpartum (Table 1). Nineteen percent of all mothers

tested postnatally (n=200) had Hb <12 g/dL and 35% had ferritin <12 mcg/L, the international lower reference for iron status of non-pregnant women.³

Most mothers had an adequate serum folate concentration antenatally, and therefore folate levels were not checked in the postnatal period. Serum folate was low (<3 ng/mL) in 3% of all mothers antenatally. Anemic mothers had mean (SD) 8.2 (4.6) ng/mL serum folate (n=103) and non-anemic controls had mean 10.7 (5.8) ng/mL (n=120) ($P=0.001$), also related to the number of prescriptions for hematinics.

Serum B₁₂ was low (<200 pg/mL) in 67% of mothers antenatally (n=224), distributed evenly between the groups. Twenty-six percent of mothers had antenatal serum B₁₂ values <100 pg/mL, with 10% at 50 pg/mL or below. B₁₂ levels of those mothers with low values were checked at 6 months postpartum, when 27% had values <200 pg/mL, 10% <150 pg/mL and 2% <100 pg/mL (n=127). Distribution of low values was 21% in the subject group (n=58) and 32% in controls (n=69). A follow-up at 12 months postpartum found 19 out of 31 mothers with persistently low values: 61% <200 pg/mL and 6% <100 pg/mL (n=31). Mothers who became pregnant within the year were not re-tested. One mother reported neurological symptoms postnatally, which improved with vitamin B₁₂. A strong significant correlation was found between maternal B₁₂ levels during pregnancy and serum B₁₂ at 6 months postpartum ($r=0.4$; $P \leq 0.0005$) (n=123). However, only those mothers with low values were assessed postnatally. There was no correlation between values at 6 and 12 months postpartum ($r=-0.096$; $P=0.6$) (n=28). Correlation was positive but not significant between antenatal values and those at 12 months postpartum ($r=0.3$; $P=0.1$) (n=30).

Low B₁₂ levels were not associated with anemia or macrocytosis. The mean MCV of the anemic mothers was consistently lower than the reference value antenatally and at 6 months postpartum (Table 1). There was a strong correlation between antenatal and postnatal values for MCV ($r=0.85$) and MCH ($r=0.82$; $P \leq 0.0005$) (n=195). The mean (SD) red cell count was low in anemic mothers antenatally at 3.9 (0.4) (n=107) and at the lower limit of normal at 4.2 (0.4) in controls (n=124) (Table 1), but was normal postnatally, suggesting the effects of hemodilution in pregnancy. Diminished hematopoiesis is also an outcome of folate or B₁₂ deficiency, through reduced DNA synthesis and the formation of megaloblastic cells in the bone marrow, with sufficient cytoplasm but insufficient chromatin to divide.²⁰

Low birthweight infants (<2500 g) made up 4.4% of the sample, distributed evenly between the two groups. Hemoglobin at birth and throughout the year was lower than reference values^{3,7} (Table 2) (detailed hematology¹⁸).

The incidence of iron-deficiency anemia between 6 and 12 months was 72%, significantly higher at 81% (n=91) for infants born to anemic mothers, compared with 65%

for controls (n=112), ($P=0.01$). While blood test results indicated iron-deficiency anemia, clinically these infants were healthy. From 9 months of age, approximately 25% of infants demonstrated an increased dependence on breastfeeding and refusal of weaning foods which had been accepted earlier. While this coincided with an increase in iron deficiency, it was not restricted to anemic infants.

TABLE 3. Aspects of fetal outcome/infant welfare in relation to maternal anemia[†].

Mean	Subject Group	Control Group	P-value
Birthweight (g) (SD)	3308 (515) (n=107)	3258 (488) (n=125)	NS
Low birthweight (%)	4	5	NS
Gestation (weeks) (SD)	40.1 (1.0) (n=107)	39.8 (1.0) (n=125)	NS
Weight at 12 months (g) (SD)	9224 (1191) (n=86)	9328 (1008) (n=96)	NS
Weight gain (0-12 months) (g) (SD)	5916 (1137) (n=86)	6070 (944) (n=96)	NS
Infection rates (0-12 months)			
Acute respiratory infection (SD)	26.7 (21) (n=89)	27.7 (23) (n=108)	NS
Diarrheal disease (days) (SD)	7.8 (12) (n=89)	5.9 (8) (n=108)	NS
Iron deficiency anemia (%)	81 (n=91)	65 (n=112)	0.01

[†] *t*-test for means of independent groups, 2-tailed significance, SPSS.

Table 3 shows aspects of fetal outcome/infant welfare in relation to maternal anemia, indicating no significant clinical adverse outcome for the infants born to anemic mothers. However, developmental tests were not performed in the study.

Most of the anemia in infancy could be diagnosed as related to iron deficiency, using research criteria, with a corresponding microcytic, hypochromic blood film (Table 2). However, the diagnosis was sometimes ambiguous. In 11% of the anemic infants (22 cases), there was no clear indication of iron deficiency. These infants were of normal birthweight and 68% were of male gender. Maternal characteristics of this group included 36% maternal iron-deficiency anemia (36% of the subject group), 68% incidence of low serum B₁₂ <200 pg/mL and a normal serum folate in pregnancy. Some infants had both low MCV and MCH with anemia, while plasma ferritin and ZPP were within normal limits. Also in some cases of iron-deficiency anemia, classified by low hemoglobin and plasma ferritin, MCV and MCH were within normal range: 15% of these infants had MCV >73 fl and 19% had MCH 24 pg or above (n=203). This may reflect different stages of iron deficiency in the infants, or the combined effect of other nutritional deficiencies,^{4,21,22} including vitamin B₁₂ or folate, which may present as macrocytosis.

Discussion

Mothers in developing countries often embark on pregnancy with low iron and other nutritional stores,

through the combined effects of inadequate nutrition and a cycle of pregnancy and lactation in their childbearing years. Recent surveys in Jordan support this.^{1,5,6} A 23% prevalence of anemia in non-pregnant women of childbearing age was reported by UNRWA in 1990,¹ while in 1996, the Ministry of Health found a 29% prevalence, rising to 38% in rural areas.⁶ In Amman Governorate, 53% had low iron stores, indicated by serum ferritin <12 mcg/L.^{3,6} Prevalence of anemia increased in late pregnancy to 42%,⁶ and to 53.7% in the UNRWA study.¹

Mothers in Jordan traditionally tend to have several children close together in the early years of marriage, and to space pregnancies later, many relying on the contraceptive properties of breastfeeding. Ninety percent of the mothers in our study were breastfeeding at 6 months postpartum, and 60% at one year, a pattern representative of national practice.²³ The 1994 National Census described a high fertility rate for Jordan of 4.6, and an average birth interval of 14 and 15 months has been reported.^{23,24} Twenty-six percent of the mothers in our study conceived with a birth interval of less than one year. A short birth interval may jeopardise the pregnancy and compromise recovery and replenishment of maternal stores.^{22,25,26} Anemic mothers in our study had a significantly lower hematocrit at "booking" than controls, suggesting a compromised nutritional status early in pregnancy. Low hemoglobin and plasma ferritin values persisted at 6 months postpartum.

The anemic mothers also had a significantly lower serum folate level antenatally than controls (Table 1). It is estimated that without folic acid supplementation, megaloblastic bone marrow changes will be found in 20%-60% of women in late pregnancy.²⁷ Folate deficiency has been associated with pre-term birth, low birthweight²⁸ and with congenital neural tube defects.²⁹ Low levels in lactating women may be reflected in low breast milk folate.²⁷ However, only 3% of mothers had low folate levels of <3 ng/mL. The Jordanian diet is rich in leafy salads, containing folate, but traditional re-heating of cooked foods destroys some available folic acid. Folate supplements were also prescribed with iron at the clinic. No other data on maternal folate or B₁₂ levels in Jordan was available for comparison. While folate deficiency is a common feature of pregnancy, our findings of an adequate maternal folate status agree with those of studies in northern Pakistan⁹ and rural Mexico.¹⁰

The majority of mothers had a low serum B₁₂ value at term. This is a phenomenon of pregnancy, thought to be partly related to hemodilution, and partly a disturbance in cobalamin metabolism: low serum levels may not reflect low tissue B₁₂ levels.²⁰ However, low values persisted at 6 months and at 12 months postpartum. Low B₁₂ levels were not related to anemia or to macrocytosis, as may be anticipated if erythropoiesis were impaired through B₁₂ or folate deficiency, although this may be a later

development, preceded by megaloblastic changes in bone marrow.²⁰

Other authors have reported low B₁₂ values in developing countries in the population in general⁸⁻¹⁰ and in mothers during pregnancy and lactation and their infants in particular.¹⁰⁻¹⁵ When maternal B₁₂ status is low during pregnancy and lactation, fetal storage may be suboptimal and breast milk content reduced.^{10-13,22} In northern Pakistan, serum B₁₂ levels in a healthy population were considerably lower than those described in the literature.⁹ Low B₁₂ values in rural Mexico were attributed to malabsorption of B₁₂ associated with *Giardia*.^{10,22} A 1994 national survey in Jordan³⁰ reported *Entamoeba coli* in 14.3% and *Giardia* in 8.4% of stool samples of school-children, and these may also reduce absorption of B₁₂.

Anemia in pregnancy was found in this study to be significantly related to the incidence of iron-deficiency anemia in infancy.¹⁸ It could be argued that since the infants were clinically healthy, the high prevalence of anemia and iron deficiency in infancy reported here only represents an altered laboratory profile: no adverse effect has been demonstrated from being born to an anemic mother. However, development screening was not performed in our study. Developmental delay in anemic infants has been widely documented.¹⁴⁻¹⁷ While the high incidence of anemia in the Jordanian infants was mainly diagnosed as due to iron deficiency, 11% of infants had an ambiguous hematology, which may have reflected a combination of nutrient deficiencies,^{4,21} and may have been associated with the low serum B₁₂ values found in mothers up to one year after delivery. Maternal supplementation with vitamin B₁₂ during pregnancy and lactation may be necessary in some communities, in addition to current iron and folic acid, to benefit both mother and infant.

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

References

1. Cook R. UNRWA Annual Report of the Director of Health. Vienna: United Nations Relief and Works Agency for Palestinian Refugees in the Near East, 1990.
2. World Health Organisation (WHO). Nutritional anaemias: report of a WHO scientific group. Technical Report Series, No. 405, 1968.
3. International Nutritional Anaemia Consultative Group (INACG). Measurement of Iron Status. Washington DC: The Nutrition Foundation, 1985.
4. Yip R. Iron-deficiency: contemporary scientific issues and international programmatic approaches. Symposium: Clinical nutrition in developing countries. J Nutr 1994;124:1479S-1490S.
5. Jilani I, Qazaq HS, Al-Arabi ZA. A study on anemia among pregnant at Mother and Childhood (MCH) Centres in Jordan for the year 1990 and the first half of 1991. Ministry of Health, Jordan, 1992.
6. Mawajdeh S, Badran O, Haddadin A, Abu-Laban A, Idris M. Prevalence and determinants of anaemia and iron-deficiency among Jordanian women 15-49 years of age: a national study. UNICEF, Jordan Country Programme and the Ministry of Health, Jordan, 1996.
7. Dallman PR, Yip R, Oski FA. Iron deficiency and related nutritional anemias. In: DG Nathan and FA Oski. Hematology of infancy and childhood. 4th edition. Philadelphia: Saunders Company, 1993.
8. Chanarin I, O'Shea AM, Malkowska V, Rinsler MG. Megaloblastic anaemia in a vegetarian Hindu community. Lancet 1985;2:1168-72.
9. Modood-ul-Mannan, Anwar M, Saleem M, Wiqar A, Ahmad M. A study of serum vitamin B₁₂ and folate levels in patients of megaloblastic anaemia in Northern Pakistan. J Pak Med Assoc 1995;45:187-8.
10. Allen LH, Rosado LR, Casterline JE, Martinez H, Lopez P, Munoz E. Vitamin B₁₂ deficiency and malabsorption are highly prevalent in rural Mexican communities. Am J Clin Nutr 1995;62:1013-9.
11. Almadan MS, Al Awamy BH, Al Mulhim IA. Nutritional B₁₂ deficiency in infancy. Indian J Pediatr 1993;60:683-701.
12. Specker B, Black A, Allen L, Morrow F. Vitamin B₁₂: low milk concentrations are related to low serum concentrations in vegetarian women and to methylmalonic aciduria in their infants. Am J Clin Nutr 1990;52:1073-6.
13. Neumann CG, Harrison GG. Onset and evolution of stunting in infants and children. Examples from the Human Nutrition Collaborative Research Support Program. Kenya and Egypt studies. European J Clin Nutr 1994;48:S90-102.
14. Walter T, De Andraca I, Chadud P, Perales CG. Iron deficiency anemia: adverse effects on infant psychomotor development. Paediatrics 1989;84:7-17.
15. Lozoff B, Wolf AW, Jimenez E. Iron deficiency anemia and infant development: effects of extended oral iron therapy. J Pediatr 1996;129:382-9.
16. De Andraca I, Walter T, Castillo M, Pino P, Rivera P, Cobo C. Iron deficiency anemia and its effects upon psychological development at preschool age: a longitudinal study. Nestlé Foundation Annual Report, 1990;53-62.
17. Sadowitz PD, Livingston A, Cavanaugh RM. Developmental regression as an early manifestation of vitamin B₁₂ deficiency. Clin Pediatr 1986;25:369-71.
18. Kilbride J, Baker TG, Parapia LA, Khoury SA, Shuqaidef SW, Jerwood D. Anaemia during pregnancy as a risk factor for iron-deficiency anaemia in infancy: a case-control study in Jordan. Int J Epidem 1999;28:461-8.
19. Colomer J, Colomer C, Gutierrez D, Jubert A, et al. Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) Study. Paediatr Perinat Epidemiol 1990;4:196-204.
20. Amos RJ, Dawson DW, Fish DI, Leeming RJ, Linnell JC. Guidelines on the investigation of cobalamin and folate deficiencies. Clin Lab Haemat 1994;16:101-15.
21. Filteau S. Iron nutrition in children under five years old: requirements and assessment. In: Nestel P. Iron interventions for child survival. Proceedings of Opportunities for Micronutrient Interventions (OMNI) meeting in London, USAID, 1995.
22. Allen LH. Vitamin B₁₂ metabolism and status during pregnancy, lactation and infancy. In: Allen LH, King J, Lönnerdal B. Nutrient regulation during pregnancy, lactation and infant growth. New York: Plenum Press, 1994.
23. Jordan Population and Family Health Survey, Department of Statistics and Ministry of Health, Jordan, 1990.
24. Al-Qutob R, Hijazi S, Bashir N, Bustami K. Iron stores in poorer Jordanian women and their newborns. Postgrad Doc Middle East 1992;15:300-4.

25. Viteri F. The consequences of iron deficiency and anemia in pregnancy. In: Allen LH, King J, Lönnerdal B. Nutrient regulation during pregnancy, lactation and infant growth. New York: Plenum Press, 1994.
26. Winkvist A, Rasmussen KM, Habicht JP. A new definition of maternal depletion syndrome. *Am J Public Health* 1992;82:691-4.
27. Food and Agriculture Organisation of the United Nations. Requirements of vitamin A, iron, folate and vitamin B₁₂. Report of a joint FAO/WHO expert consultation, Rome, 1988.
28. Scholl TO, Hediger ML, Schall JI, Khoo CS, Fischer RL. Dietary and serum folate: their influence on the outcome of pregnancy. *Am J Clin Nutr* 1996;63:520-5.
29. Wald NJ, Hackshaw AK, Stone R, Sourial NA. Blood folic acid and vitamin B₁₂ in relation to neural tube defects. *Br J Obstet Gynaecol* 1996;103:319-24.
30. Shuqaidef SW. Study of intestinal parasites and anemia in 3rd, 4th and 5th grade school-children in Jordan. United Nations Children's Fund/ Ministry of Health, Jordan, 1995.
31. Kilbride J, Baker TG, Parapia LA, Khoury SA. Incidence of iron-deficiency anaemia in infants in a prospective study in Jordan. *Eur J Haematol* 2000;64:231-6.