Original Article

Zinc and iron status during pregnancy of Filipino women

Nynke de Jong PhD, Aurora B Ampong Romano MD, MPH and Rosalind S Gibson PhD

Department of Human Nutrition, University of Otago, Dunedin, New Zealand

Low birthweight is associated with maternal anaemia and, in some circumstances, with low iron and zinc status, but this relationship has not been investigated in the Philippines. In this study, we assessed the prevalence of anaemia and suboptimal iron and zinc status in pregnant women from three geographical regions (mountain, coast, city) of Zamboanga del Sur province at 24 weeks ($n = 305$), and again at 36 weeks ($n = 127$), gestation. At 24 weeks, 34% were anaemic (i.e., haemoglobin < 105 g/L) from all causes, of whom only 14% had concomitant low serum ferritin values (i.e., < 12 µg/L). The presence of infection was low, based on both elevated white blood cell count ($> 11 \times 10^9$/L; 19%) and serum C-reactive protein (> 15 mg/L; 3%). Of the women surveyed, 20% were iron depleted but not anaemic, and 15% were zinc deficient (i.e., serum zinc < 7.1 µmol/L). The mean ($\pm$ SD) birthweight of the infants ($n = 250$) was 3074 g $\pm$ 408 g, of whom 5% were of low birthweight (< 2500 g). No differences existed for biochemical indices or birthweight among the three regions, or between women consuming maize or rice-based diets. Women with low haemoglobin ($P = 0.05$) and low serum zinc ($P = 0.14$) values at 24 weeks gestation had infants with lower birthweights than those with values ≥ 105 g/L and ≥ 7.1 µmol/L, respectively. However, in the multivariate model, the contribution of maternal haemoglobin to the variance in birthweight at 24 weeks gestation was non-significant, although modest for serum zinc. Anaemia and/or suboptimal zinc status during pregnancy may be related to low birthweight in the Philippines, and their aetiology deserves further study.

Key words: anaemia, birthweight, haemoglobin, hair, iron, Philippines, pregnancy, Zamboanga del Sur, zinc.

Introduction

The prevalence of anaemia among pregnant women in the Philippines is high. Indeed, between 1993 and 1998, national levels actually increased from 44 to 51% according to the fourth and fifth Philippine National Nutrition Surveys.[1,2] In 1995, the World Bank reported an infant mortality rate of 57 per 1000 live births in the Philippines, which was classified among the highest in South-east Asian countries.[3] In addition, a persistently high incidence of low birthweight and prematurity has been reported. According to the latest provincial data, the prevalence of low birthweight in the province of interest, Zamboanga del Sur, was 12.1% in 1999 and 9.6% in 2000 (Field Health Services Information System, Ministry of Health, Manila, unpubl. data).

Anaemia due to iron deficiency is reported to be the most common form of malnutrition in the Philippines.[3] Pregnant women are especially vulnerable to iron deficiency anaemia (IDA), because of their increased need for iron for the development of the foetus and placenta, and the expansion of maternal red cell mass. As a result, iron supplementation programs for pregnant women are among the most widespread national public health programs.[4] Requirements for iron rise progressively during pregnancy, so that in the second and third trimesters, estimated increases are 4 and 10 mg/day, respectively. To meet these high iron needs and maintain iron balance throughout pregnancy, women should have adequate stores of iron at the onset of pregnancy.[5] However, very few

pregnant women in developing countries have sufficient iron stores at this critical stage. Indeed, even in Western countries, only 20% of pregnant women meet the lower limit of their requirements for iron.[3,6]

Concomitant with this increased need for iron during pregnancy is an elevated requirement for zinc, due to its critical role in the growth and development of the foetus and maternal tissue accretion.[7] Several studies, both cross-sectional and zinc-supplementation trials in developed and developing countries, have investigated the relationship between maternal zinc status and pregnancy outcome. Results have been inconsistent and have been reviewed in detail by King.[8] Such inconsistencies across studies have been attributed in part to problems with the collection, analysis and interpretation of serum/plasma zinc concentrations, as well as the numerous factors affecting birthweight besides maternal zinc status.

The aetiological factors associated with suboptimal maternal zinc status have also been linked with poor iron status: plant-based diets that contain strong inhibitors of zinc

Correspondence address: Dr Rosalind S Gibson, Department of Human Nutrition, University of Otago, PO Box 56, Dunedin, New Zealand.
Tel: +64 3479 7955; Fax: +64 3479 7958
Email: rosalind.gibson@stonebow.otago.ac.nz

and non-haem iron absorption, low intakes of flesh foods that are rich sources of readily available iron and zinc, multiparity and parasitic infections.[9,10] Geographic factors such as a low soil zinc content[11] and a hot humid climate that induces increased endogenous losses through perspiration and exfoliation of the skin, may exacerbate maternal zinc and iron deficiency.[3]

In light of the high prevalence of anaemia in pregnant Filipino women and the concordance between the aetiological factors associated with iron and zinc deficiency, we hypothesised that the zinc status of pregnant Filipino women may also be compromised. However, to our knowledge, no studies focusing on zinc status in pregnant Filipino women have been published. Also, despite the high prevalence of anaemia, its association with pregnancy outcome has received little attention in the Philippines. Therefore, the current study aimed to assess (i) the prevalence of a suboptimal iron and zinc status in a group of pregnant Filipino women residing in three different geographical areas; and (ii) the relationship between maternal iron and zinc status at 24 and 36 weeks gestation, and infant birthweight.

Subjects and methods

The study was carried out from October 1999 to May 2000 in three different geographical areas (city, mountain, coast) in the province of Zamboanga del Sur, the Philippines. The survey areas were selected on the basis of the 1995–97 health statistics of the province that indicated a high prevalence of premature births. Additional factors influencing the selection of the areas were perceived willingness of rural health personnel and local officials, as well as accessibility of the areas. In total, four main health centres in the provincial capital Pagadian City, six municipalities in the mountainous area and nine coastal municipalities participated in this study.

Between October and December 1999, all pregnant women who were eligible for the study were invited to participate through selected health centres and municipalities. All women, with the exception of two, signed the study consent form and participated in the regular antenatal care program. Since 1996, the services in this program have included monitoring anthropometric variables (height, weight, fundal height) and blood pressure, as well as the provision of a daily iron supplement for 120 days, from the beginning of the second trimester of pregnancy until six weeks after delivery. Anaemic women are supplied with two tablets daily, each containing 60 mg iron as ferrous sulphate and 400 μg folate; non-anaemic women receive one tablet per day. The tablets are given out free of charge by midwives at the prenatal clinics held in the local health centres. Women are requested to visit the clinics once every four weeks until seven months into the pregnancy. During the seventh and eighth months, they are expected to attend biweekly, and then weekly during the last month. Quantitative data on the number of women in this study receiving iron/folate supplements from the health centre and the dose received were not available.

Ethical approval for the conduct of this study was obtained from the Human Ethics Committee of the University of Otago, Dunedin, New Zealand, the Provincial Health Board of Zamboanga del Sur, Philippines, and the city mayor of Pagadian City, Philippines.

Participants

For phase I of the study, a total of 305 pregnant women at 24.1 ± 0.6 weeks of gestation (mean ± SD) were identified by trained health personnel (midwives, nurses and the co-investigator ABAR) from the health centres. Gestational age was determined by the women's recalled date of their last menstrual period (LMP; $n = 294$). In cases when the latter was unknown ($n = 11$), fundal height was used to calculate gestational age.[12] Consenting women were included in the study if they were aged between 15 and 45 years, and if they had a normal, single pregnancy. Anthropometric, biochemical, general health and sociodemographic data were obtained at 24 weeks of gestation. Self-reported data were collected on prenatal dietary supplement use and prepregnancy weight. Health personnel registered birth outcome and also measured infant birthweight after delivery.

For phase II of the study, a convenience sample of 127 women was selected from the initial sample of 305 women. A second nutritional assessment involving anthropometric, biochemical and clinical assessment was performed on this subsample at 36 weeks of gestation. Birth outcome and infant weight after delivery were also obtained in this subsample.

Biochemical assessment and blood pressure

Two fasting, peripheral venipuncture blood samples (7.5 mL in a trace element-free vacutainer without coagulant (Becton Dickinson, Rutherford, NJ, USA) and 5 mL in an ethylenediaminetetraacetate (EDTA) vacutainer were collected from all women at 24 weeks of gestation and in a subsample of women at 36 weeks of gestation ($n = 127$) by trained health personnel. Blood samples were transported in a cool box with ice packs to the laboratory within 6 h of collection. Haemoglobin analyses (cyanmethemoglobin method) and white blood cell count (WBCC) were performed immediately after arrival in the provincial hospital clinical chemistry laboratory. Serum was separated from the red blood cells using trace element-free techniques, and then shipped under dry ice to New Zealand, where they were stored at –20°C for analysis in the trace element laboratory of the Department of Human Nutrition, University of Otago.

Serum ferritin was assayed by an enzyme-linked immunosorbent assay (ELISA) procedure using a commercial kit (Ramco Laboratories, Houston, TX, USA). Serial replications of quality control sera for serum ferritin were used to check the precision and accuracy of the assay. The mean ± SD values of the control sera, 12.9 ± 0.88 μg/L, 68.0 ± 5.6 μg/L and 292.9 ± 22.0 μg/L, were within the manufacturer's specified acceptable ranges of 12.6 ± 5 μg/L, 64.4 ± 12.4 μg/L and 281 ± 47 μg/L, respectively. The level of C-reactive protein in serum was measured by kinetic turbidimetry using the Behring Turbitimer (Behringwerke AG, Marburg, Germany). Serum zinc was measured by flame atomic absorption spectrophotometry (AAS; Analyst800,

Perkin-Elmer, Norwalk, CT, USA) using a modification of the method described by Smith and colleagues.[13] Serial replication of aliquots from a pooled serum sample and quality control sera were used to check the precision and accuracy of the analytical method for zinc. The within-run coefficient of variation (CV) for zinc in a pooled serum sample was between 2.2 and 4.0%, based on six to seven samples in each of the five runs. The mean ± SD value (%CV) for the quality control sera (Bovine serum reference material no. 1598, National Institute of Standards and Technology, Gaitherburg, MD, USA) was 13.8 ± 0.5 µmol/L (3.5%; n = 15) compared with the certified value of 13.6 ± 0.1 µmol/L.

Hair samples were collected from the occipital portion of the scalp with stainless steel scissors. Only the proximal 1.0–1.5 cm of the hair strands were retained, washed by a modification of the non-ionic detergent procedure of Harrison *et al.*,[14] acid digested with ultra pure nitric acid (70%), then analysed for zinc by flame AAS. The %CV for zinc in aliquots of a pooled powdered hair sample was 1.9% (n = 19). Values of aliquots of a certified reference material for human hair (Commission Bureau of Reference, Reference Material no. 152) were: mean ± SD (%CV) 3.07 ± 0.05 µmol/g (1.6%) compared to the certified value of 3.04 ± 0.08 µmol/g. The samples were analysed in two runs with a mean difference of 0.75% between seven duplicate samples.

The blood pressure of the women was measured at 24 and 36 weeks of gestation in a subsample of the women by health personnel, following standard procedures.

Anthropometry

Selected anthropometric measurements were carried out in duplicate on the women at 24 weeks of gestation and on a subsample at 36 weeks of gestation. Height (cm) was measured with a stadiometer to the nearest 0.5 cm. Weight (kg) was measured to the nearest 0.1 kg, with women wearing light clothing and no shoes and using a beam balance with non-detachable weights. Mid-upper arm circumference (MUAC) was measured via a fibreglass insertion tape according to standardized techniques.[15] Fundal height was recorded using tape measures.[12] Data on prepregnancy weights were not available. As a result, self-reported prepregnancy weights were recorded.

From delivery up to a maximum of 5 days after delivery, the birthweights of the infants were measured by the health personnel in charge. Each child was weighed to the nearest gram on a spring balance weighing scale (Salter Abbey Weighing Machines, Suffolk, UK), ranging from 0 to 5 kg after removing all clothing and diapers.

Questionnaires

A general questionnaire, containing questions on age, sociodemographic variables, use of vitamin and mineral supplements and medications, medical and obstetrical history and, for the subsample, type of diet (i.e., maize or rice as a staple food) was completed by each woman and collected by the trained health personnel.

Statistical methods

Statistical analyses were performed using SPSS for windows, version 9.0 (SPSS, Chicago, IL, USA). All variables were tested for normality by using Kolmogorov Smirnov tests. However, in cases where results were uncertain, visual inspection and a priori assumptions about normality were also applied. The results of those variables shown to be distributed normally are expressed as mean ± SD, and for those with skewed distributions, as the median $(P_{25}–P_{75})$. Differences among the three geographical groups or among the different supplement-using groups (i.e., iron only, iron plus folate, multivitamins or none) for continuous normally distributed variables were tested using one-way analysis of variance (ANOVA) followed by post hoc comparisons (Bonferroni method), whereas for continuous, non-normally distributed variables, the Kruskal–Wallis test was used. Differences between the two dietary groups in the subsample (i.e., rice and maize eaters, based on their main staple), were tested using a *t*-test for normally distributed variables, or the Mann–Whitney *U*-test when necessary. Differences between biochemical and anthropometric variables in the subsample measured at 24 and 36 weeks of gestation were tested with Wilcoxon signed ranks tests. Associations were explored with Spearman's rank correlation tests. A *P*-value ≤ 0.05 was considered to be significant. To assess the contribution of zinc and iron parameters to the variance in birthweight, multiple regression analyses were performed. The choice of confounding parameters (i.e., type of diet, infection measures, maternal bodyweight, maternal MUAC, blood pressure, chronological age, parity, iron supplement or multivitamin use, geographical area, sex of the infant) was based on earlier evidence from the literature.[4,8]

Results

Of the 305 women enrolled, 302 completed a general questionnaire. Most of the women were homemakers (n = 261; 86%) with some form of formal education (n = 301); either elementary (n = 100; 33%), high school (140; 46%), or college or professional level (n = 61; 20%). Of the women who participated in the study, 39% (n = 118) were nulliparous, 23% (n = 69) primaparous, 18% (n = 54) were parity two, 9% (n = 28) were parity three and only 11% (n = 32) were parity four or more. The religion of the women was predominantly Bisaya (n = 199), followed by Subanen (n = 27), Muslim (n = 12) and other religions (n = 63). The dietary supplements reportedly taken by the women at 24 weeks gestation were iron and folate (33%; n = 100), followed by iron only (27%; n = 82) and then multivitamin tablets (19%; n = 59); 19% (n = 57) consumed no dietary supplements.

Both the regional distribution, and the demographic and socioeconomic characteristics of the participants in phase II were comparable to those of phase I. Of the phase I participants, 20% were from the city, 50% were from the coast and 30% were from the mountainous area, compared to 28, 48 and 24%, respectively, in the phase II subsample. The age of the women (mean ± SD) was 27.3 ± 7.2 years in phases I and 27.6 ± 6.7 years in phase II. In relation to years of schooling,

the percentages of the women in phase II were comparable to the percentages found in phase I. For example, of the phase II women, 54% (compared to 46% in phase I) had attended high school, 27% (compared to 33% in phase I) had attended elementary school and 18% (compared to 20% in phase I) had attended college or were classified as professional. Most of the women in phase I and II were homemakers (86 *vs* 89%) who lived with their immediate family (74 *vs* 73%) and had a relatively low income (between 1000 and 6000 pesos/month).

Anthropometric measurements were obtained from 274 women at 24 weeks of gestation. Infant birthweights were recorded for a total of 254 women. Of these, three delivered a set of twins and one had an infant with a congenital anomaly, and were excluded from further data analyses. The main baseline characteristics of the study sample ($n = 301$) are presented in Table 1.

With respect to the biochemical iron indices and serum zinc results at 24 weeks of gestation, no relevant differences existed among the three geographical areas (Table 2), nor

between the rice versus maize eaters (data not shown) in the subsample. The prevalence of biochemical parameters below specific cut-off levels was not significantly different among the three areas either (Table 3). Of the total group, 34% had serum ferritin concentrations less than 12 µg/L, with or without anaemia. Likewise, in the whole group, the prevalence of anaemia was also 34% (with or without low serum ferritin values), based on a cut-off value for haemoglobin specific for the second trimester (i.e., <105 g/L). However, when the non-gestational appropriate WHO cut-off value of 110 g/L[16] was applied, 51% of the women were classified as anaemic. Hemoglobin and serum zinc were significantly correlated at 24 weeks ($r = 0.22$; $P < 0.001$; $n = 271$), but not at 36 weeks of gestation ($r = 0.14$; $P < 0.21$; $n = 84$). Of the participants, a total of 19% at 24 weeks and 15% at 36 weeks had a WBCC above 11×10^9/L, whereas 3 and 2% had a serum CRP > 15 mg/L at 24 and 36 weeks, respectively, levels said to be indicative of infection.[17] Cross-tabulation revealed that a total of 14 subjects had an elevated WBCC consistent with anaemia.

Table 1. Characteristics of the total study sample at study entry, classified according to geographical area

Parameter	Total group	Mountain	Coast	City	*P*-value
Age (year)	27.3 ± 7.2 (301)	27.0 ± 7.8 (91)	27.4 ± 7.1 (151)	27.5 ± 6.5 (59)	0.88
Gestational age (week)	24.1 ± 0.6 (298)	24.3 ± 0.6 (88)	24.0 ± 0.7 (147)	24.3 ± 0.6 (59)	0.52
Fundal height (cm)	18.8 ± 3.4 (291)	20.5 ± 3.9[a] (87)	18.3 ± 2.9[b] (147)	17.7 ± 2.9[b] (57)	< 0.001
Reported prepregnancy weight (kg)	49.3 ± 6.1 (274)	49.3 ± 6.6 (74)	49.2 ± 5.6 (145)	49.6 ± 6.9 (55)	0.93
Weight (kg)	53.1 ± 7.3 (291)	52.4 ± 7.1 (88)	53.3 ± 7.6 (146)	53.3 ± 7.0 (57)	0.62
Height (cm)	152.8 ± 7.6 (274)	150.4 ± 6.3[a] (88)	154.7 ± 7.5[b] (138)	152.8 ± 7.6[a] (48)	< 0.001
BMI (kg/m^2)	22.8 ± 3.1 (274)	23.2 ± 2.8 (88)	22.3 ± 3.2 (138)	23.3 ± 3.4 (48)	0.07
MUAC (cm)	25.3 ± 3.0 (288)	24.2 ± 3.5[a] (88)	25.7 ± 2.6[b] (144)	26.1 ± 2.7[b] (56)	< 0.001
Blood pressure	(292)	(88)	(147)	(57)	
Systolic (mmHg)	100 ± 11	99 ± 10	100 ± 11	101 ± 9	0.58
Diastolic (mmHg)	68 ± 8	67 ± 8	68 ± 8	67 ± 8	0.64

Statistical significance (one-way ANOVA): [a,b]different superscripts represent statistically significant differences between groups. Values are presented as mean ± SD (*n*). BMI, body mass index; MUAC, mid-upper arm circumference.

Table 2. Biochemical results of the total study sample at 24 weeks of gestation, classified according to geographical area

Parameter	Total group	Mountain	Coast	City	*P*-value
Haemoglobin (g/L)					
Median	110	110	114	110	0.23
P$_{25}$–P$_{75}$	89–120	100–120	98–125	100–117	
n	287	87	142	58	
Serum ferritin (µg/L)					
Median	15.7	17.8	15.2	15.6	0.63
P$_{25}$–P$_{75}$	9.9–29.6	10.3–32.9	9.9–25.7	8.8–28.9	
n	282	82	142	58	
Serum zinc (µmol/L)					
Median	8.7	8.8	8.7	8.8	0.65
P$_{25}$–P$_{75}$	7.6–9.8	7.8–10.1	7.6–9.6	7.4–10.6	
n	282	84	140	58	
Hair zinc (µmol/g)					
Median	2.49	2.34	2.56	2.89	0.001
P$_{25}$–P$_{75}$	2.15–3.19	2.08–2.81	2.20–3.41	2.33–3.46	
n	282	89	142	51	

Statistical significance (Kruskal–Wallis test): *P*-values ≤ 0.05 were considered significant.

The reported dietary supplement usage during phase II was comparable to that of phase I. Iron plus folate (30%) or iron only (30%) supplements were used most frequently, followed by multivitamins (25%). Of the phase II women, 13% consumed no dietary supplements.

When women were classified into four groups according to their reported use of dietary supplements (i.e., iron only, iron plus folate, multivitamins, no supplement use), no significant differences existed among the groups for birthweight or biochemical parameters for the phase I women at 24 weeks of gestation, nor for the phase II women at 36 weeks of gestation. By contrast, when women were classified on the basis of their reported use of iron supplements only compared to no use of supplements, haemoglobin at 24 weeks of gestation (but not serum ferritin at 24 or 36 weeks, or haemoglobin at 36 weeks of gestation) was significantly higher in those who reportedly used iron supplements compared to those who did not (mean haemoglobin values $\pm$ SD: 114 ± 19 vs 105 ± 17 g/L; one-way ANOVA; $P = 0.02$).

The differences in maternal biochemical and anthropometric parameters between 24 and 36 weeks of gestation are presented in Table 4. Serum ferritin concentrations decreased in all members of the group during this period (not significant), which was accompanied by a modest (but significant) decrease in haemoglobin. No significant gestational age-related differences were observed for serum zinc concentrations.

The mean birthweight in the total group (phase I) was 3074 ± 408 g ($n = 250$). The prevalence of low birthweight (i.e., <2500 g) was 5% in the total group. There were no statistically significant differences in birthweight among the three different areas, nor between the maize and rice eaters (data not shown). No significant associations between maternal weight gain and infant birthweight ($P > 0.28$), or between (changes in) biochemical iron and zinc parameters and infant birthweight ($P > 0.16$) were apparent. However, a statistically significant difference ($P = 0.05$) in birthweight (3003 g vs 3114 g) was observed when women were classified as anaemic versus non-anaemic (cut-off point of 105 g/L). Women with serum zinc values below 7.1 µmol/L tended to have infants with a lower birthweight (2965 g) compared to women with serum zinc values above the cut-off (3077 g), but this trend was not significant ($P = 0.14$). These results were not confirmed by the multiple regression model. In this model with infant birthweight as the dependent variable (Table 5), maternal weight at 24 weeks, infection via white blood cell count and systolic blood pressure were significant.

Table 3. Number and percentage of women at 24 week of gestation with iron depletion, anaemia, and zinc deficiency, classified according to geographical area†

Parameter	Total group n (%)	Mountain n (%)	Coast n (%)	City n (%)	P-value
Anaemic					
Serum ferritin <12 µg/L	39 (14)	11 (14)	20 (15)	8 (14)	
Serum ferritin ≥12 µg/L	55 (20)	17 (22)	25 (19)	13 (23)	0.76
Non-anaemic					
Serum ferritin <12 µg/L	55 (20)	17 (22)	26 (19)	12 (21)	
Serum ferritin ≥12 µg/L	122 (45)	33 (42)	65 (47)	24 (43)	0.85
Serum zinc					
<7.1 µmol/L	43 (15)	8 (10)	24 (17)	11 (19)	
≥7.1 µmol/L	239 (85)	76 (90)	116 (83)	47 (81)	0.21
Hair zinc					
<1.68 µmol/g	14 (5)	5 (6)	9 (6)	0 (0)	
≥1.68 µmol/g	268 (95)	84 (94)	133 (94)	51 (100)	0.19

†Anaemia, haemoglobin < 105 g/L at 24 week of gestation. Iron depletion, serum ferritin < 12 µg/L. Zinc deficiency, serum zinc < 7.1 µmol/dL, hair zinc < 1.68 µmol/g.[9,21,34]

Table 4. Comparison of biochemical indicators and weight and mid-upper arm circumference at 24 and 36 weeks of gestation

Parameter	n†	24 weeks Median (P_{25}–P_{75})	36 weeks Median (P_{25}–P_{75})	P-value
Haemoglobin (g/L)	89	112 (101–120)	108 (99–115)	0.04
Serum ferritin (µg/L)	96	17.8 (11.2–29.4)	14.9 (10.3–24.5)	0.08
Serum zinc (µmol/L)	96	9.4 (8.2–10.6)	9.5 (8.2–10.5)	0.99
Hair zinc (µmol/g)	233	2.49 (2.15–3.19)		
Anthropometric parameters (mean ± SD)				
Weight (kg)	91	52.9 ± 7.0	56.6 ± 7.2	< 0.001
MUAC (cm)	88	25.7 ± 2.4	26.5 ± 3.5	0.26

†Numbers vary because birthweight records were not available for all infants. Statistical significance (Wilcoxon signed ranks test): P-values ≤ 0.05 were considered significant. MUAC, mid-upper arm circumference.

Table 5. Regression model with infant birthweight as the dependent variable

Independent variables	Standardized β-coefficient	t	P-value
Maternal weight	0.157	2.239	0.026
White blood cell count	− 0.182	− 2.655	0.009
Serum zinc	0.130	1.821	0.070
Systolic blood pressure	− 0.166	− 2.399	0.017
Haemoglobin	− 0.031	− 0.430	0.668

Independent variables were measured at 24 weeks. Linear regression model r^2 = 0.095. P-values ≤ 0.005 were considered significant.

Serum zinc at 24 weeks approached significance (P = 0.07), but haemoglobin, as well as other confounding variables, did not contribute significantly.

Discussion

Until now there have been no studies published on the zinc status of pregnant Filipino women. Moreover, despite the high prevalence of iron deficiency anaemia among pregnant women in the Philppines,[3,18–20] no study has investigated whether pregnancy outcome is associated with IDA. In our study on pregnant women living in the province of Zamboanga del Sur, the Philippines, we found 34% of the subjects to be anaemic from all causes. An additional 20% of the women were non-anaemic, but may have had depleted iron stores based on low serum ferritin concentrations (i.e., <12 μg/L; Table 3). In our sample, 15% had low serum zinc concentrations indicative of zinc deficiency. No significant differences in the biochemical iron and zinc parameters among the three geographical areas, or between women who consumed rice and maize as their major staple, were observed. Only 5% of the infants had a birthweight below 2500 g. Women with a haemoglobin level below the cut-off of 105 g/L at 24 weeks of gestation tended to have infants with a lower birthweight (P = 0.05), compared with those with a haemoglobin level above this cut-off. However, in the multivariate model, the contribution of maternal haemoglobin to the variance in birthweight at 24 weeks gestation was non-significant, although modest, for serum zinc (Table 5). No significant relationships were observed between birthweight and maternal serum ferritin, or hair zinc concentrations.

Biochemical iron and zinc status

With respect to iron nutriture in pregnant women, current knowledge indicates that: (i) a high proportion of women develop anaemia during pregnancy; and (ii) iron deficiency anaemia is a risk factor for preterm delivery and the subsequent low birthweight of the infant.[4] Several parameters can be used to assess iron status during pregnancy, including haemoglobin, serum ferritin, transferrin saturation (based on serum iron and total iron-binding capacity) and serum transferrin receptor concentrations.[5,6] In our study, we used a two-parameter approach based on haemoglobin and serum ferritin to assess iron status. A substantial proportion of the women studied at 24 weeks of gestation were classified as anaemic, based on a trimester-specific cut-off haemoglobin value.[21] However, the majority of these women had serum ferritin values indicative of adequate iron status, which implies a non-iron-deficient anaemia. Serum ferritin may be a less valid biochemical marker of iron stores in developing countries that have a high prevalence of infection. Concentrations may rise in the presence of infection because ferritin acts as a positive acute-phase reactant protein.[10,22] In our sample, however, only a small number of women had evidence of infection, suggesting that our apparently normal serum ferritin levels were probably not caused by infection, but instead were indicative of adequate iron status.

Several other factors, in addition to iron, may be implicated in the aetiology of the anaemia reported here. Nutrients known to have a role in erythropoiesis include iron, vitamin B_{12}, folate, riboflavin, copper and vitamin A (via its interaction with iron).[23] Deficiencies of some of these micronutrients may occur in pregnant women in the Philippines. Certainly, vitamin A deficiency is well documented in the Philippines, and recent evidence suggests that vitamin B_{12} deficiency may be more prevalent among pregnant women in developing countries than formerly appreciated.[24] Such a deficiency of vitamin B_{12} may arise from poor intestinal absorption, possibly exacerbated by low dietary intakes.[25]

In general, a decrease in biochemical iron and zinc parameters during pregnancy is explained by a disproportionate increase in plasma volume, as well as the maternal-fetal transfer.[5,26] In our study, we found that 20% of the non-anaemic women had a low serum ferritin level at 24 weeks of gestation. Furthermore, we observed a fall in serum ferritin in our subsample of pregnant women from 24 to 36 weeks of gestation, which was accompanied by a modest decrease in haemoglobin (Table 4). When interpreting these figures, some caution is warranted because of the influence of maternal plasma volume expansion, as discussed earlier. Furthermore, a decrease in serum ferritin concentration was observed, especially in women who enter pregnancy with low iron stores, sometimes irrespective of whether iron supplementation is given.[27]

In several, but not all, studies a decrease in plasma zinc during pregnancy has been reported.[8,28–31] We did not observe a decrease in serum zinc in our subgroup of women measured at 24 and 36 weeks of gestation, a finding consistent with that of some other investigators.[32,33] Tamura and colleagues suggest that after 22 weeks of gestation, serum zinc concentrations plateau.[32] Perhaps one explanation for these discrepancies is that different populations may reach a

plateau for serum zinc at different stages during pregnancy, depending on their zinc status at the onset of pregnancy.

Serum zinc concentration is used widely to assess zinc status, although its usefulness as a single parameter for defining zinc status during pregnancy is equivocal. Therefore, a combination of indices such as hair and serum zinc is often used. Hair zinc reflects chronic zinc status over a longer period of time whereas serum zinc is a more acute index of zinc status.[28] In the present study, the percentage of pregnant women with serum zinc values below the cut-off value (7.1 µmol/L) for 24 weeks of gestation[34] was 15%, less than the 43% reported for pregnant women in Malawi.[9] This cut-off value, however, was derived from non-fasting blood samples of pregnant women; therefore our prevalence figures based on fasting blood samples may be slightly underestimated. When we used 9.18 µmol/L (60 µg/dL), a value frequently used as a reference point for zinc deficiency in other pregnancy studies,[33,35] we found that 178 (63%) of our pregnant women would be classified as zinc deficient. Cross-tabulation revealed that 74 of the women below this higher serum zinc cut-off were also anaemic. When a cut-off value for hair zinc concentrations defined in children (i.e., 1.68 µmol/g) was applied, only 5% of the pregnant women were classified as zinc deficient, compared to at least 15%, based on low serum zinc concentrations. Such a discrepancy may be associated with the absence of clearly defined cut-off points for hair zinc concentrations during pregnancy.[9]

We attempted to distinguish between women who consumed diets based predominantly on rice and those whose diets were mainly maize based. We hypothesised that the women with a high maize intake would have a more unfavourable zinc status because of a higher phytate intake.[28] We failed to observe such a trend, perhaps because of the small number in our sample for whom maize was the major dietary staple. Indeed, in many cases, consumption of maize by these women was alternated with rice and as a consequence, the discrepancies in phytate intake between the two groups may not have been large enough to reveal any dietary-induced differences in biochemical zinc status.

Birthweight
A statistically significant difference in birthweight was observed when women were classified as anaemic versus non-anaemic at 24 weeks of gestation (cut-off point of 105 g/L). When women were classified according to cut-off levels for serum zinc, the difference in birthweight did not reach statistical significance. Nevertheless, in the multiple regression model, serum zinc appeared to be a stronger predictor of birthweight than haemoglobin, but the explained variance was still only very modest. Several factors may have been responsible for the modest contributions made by serum zinc and haemoglobin parameters to the explained variance in birthweight (Table 5), in addition to the relatively small sample size reported here. Possibly, the variance in birthweight was not large enough in our study sample to find clear associations. The prevalence of low birthweight was only 5% in our sample, which is low compared to provincial

data (9.6% in 2000 and 12.1% in 1999). The reason for this discrepancy is uncertain. We do not have evidence that our data are likely to be different compared to provincial data: all pregnant women registering at health centres within a certain time period were invited to participate in our study, and all except two consented, suggesting that no obvious selection bias had occurred.

Birthweight is known to be dependent on many factors besides maternal zinc and haemoglobin status, including sex of the infant, maternal age, maternal bodyweight, smoking and alcohol consumption.[4,8] We have included these factors (with the exception of smoking and alcohol consumption because so few women smoked or drank alcohol) as covariates in the regression model (Table 5), but no significant contributions were observed. Anaemia occurring in early pregnancy[4] may have a large influence on birthweight. Unfortunately, we do not have haemoglobin data before 24 weeks of gestation, but it would be of interest to study this phenomenon in the future.

The average maternal weight gain was 3.7 ± 3.6 kg over a 12-week period (0.31 ± 0.30 g/week), which is higher than the weight gain reported in pregnant women from Malawi, Central Africa,[28] but comparable to that reported in, for example, Bangladeshi women.[33] In developing countries, a weight gain of 1.5 kg per week during the last two trimesters is recommended,[36] a figure that appears to be much higher than what is currently achieved in many populations in developing countries. Such low weight gains during pregnancy have been associated with low birthweights.[37]

In conclusion, our results indicate that a substantial proportion of pregnant Filipino women are vulnerable to a poor zinc status and/or anaemia, only some of which appears to be related to suboptimal iron status. Infants born to women with a low haemoglobin level and/or low serum zinc concentration tended to have a lower mean birthweight, but the results are not conclusive. The overall prevalence of low birthweight in our sample was not alarming. The aetiological factors associated with maternal anaemia and poor zinc status during pregnancy in the Philippines deserve more attention.

References
1. Madriaga J, Cheong R, Desnacio J, Marcos J, Fuentes R, Valdez D. Philippines Fourth National Nutrition Survey. Manila: Food and Nutrition Research Institute, Department of Science and Technology, 1995.
2. Cheong RL, Madriaga JR, Perlas LA, Desnacido JA, Marcos JM, Cabrera MIZ. Prevalence of anemia among Filipinos. Phil J Nutr 2001; 48: 45–57.

3. Heaver RA, Hunt JM. Improving Early Childhood Development – An Integrated Program for the Philippines. Washington DC: World Bank, 1995.

4. Allen LH. Anaemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 2000; 71: 1280S–1284S.

5. Bothwell TH. Iron requirements in pregnancy and strategies to meet them. Am J Clin Nutr 2000; 72: 257S–264S.

6. Pilch SM, Senti FR. Assessment of the Iron Nutritional Status of the US Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976–1980. Bethesda, MA: Federation of the American Societies for Experimental Biology, 1984.

7. Gibson RS. Zinc nutrition in developing countries. Nutr Res Rev 1994; 7: 151–173.

8. King JC. Determinants of maternal zinc status during pregnancy. Am J Clin Nutr 2000; 71: 1334S–1343S.

9. Gibson RS, Huddle JM. Suboptimal zinc status in pregnant Malawian women. Its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria. Am J Clin Nutr 1998; 67: 702–709.

10. Huddle JM, Gibson RS, Cullinan TR. The impact of malarial infection and diet on the anaemia status of rural pregnant Malawian women. Eur J Clin Nutr 1999; 53: 792–801.

11. Cakmuk I, Erdal I. Phytic acid-zinc molar ratios in wheat grains grown in Turkey. Micronutr Agric 1996; 2: 7–18.

12. Farguhar C, Jamieson M. Preterm labour and delivery. Introduction to Obstetrics and Gynaecology. Auckland: Uniprint, 1994.

13. Smith JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem 1979; 25: 1487–1491.

14. Harrison WW, Yursachek JP, Benson CA. The determination of trace elements in human hair by atomic absorption spectrophotometry. Clin Chim Acta 1969; 23: 83–91.

15. Lohman TG, Roche FE, Martorell R. Anthropometric Standardization Reference manual. Champaign, IL: Human Kinetics Books, 1988.

16. Stoltzfus RJ, Dreyfuss ML. International Nutritional Anaemia Consultative Group/WHO/UNICEF. Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anaemia. Washington DC: ILSI Press, 1998.

17. Lockitch G. Handbook of Diagnostic Biochemistry and Hematology in Normal Pregnancy. Ann Arbor, MI: CRC Press, 1993.

18. Kuizon MD, Natera MG, Ancheta LP, Platon TP, Reyes GD, Macapinlac MP. Hemoglobin levels in normal Filipino pregnant women. Southeast Asian J Trop Med Public Health 1981; 12: 396–401.

19. Kuizon MD, Tajaon RT, Madriaga JR, Perlas LA, Desnaicido JA. Assessment of iron status of Filipino pregnant women. Southeast Asian J Trop Med Public Health 1989; 20: 461–470.

20. Perlas LA, Kuizon MD, Tajaon RT, Desnacido JA. Iron status of pregnant Filipino women as measured by serum ferritin. Southeast Asian J Trop Med Public Health 1992; 23: 691–696.

21. Centre for Disease Control. Criteria for anaemia in children and child bearing-age women. Morbid Mortal Week Rep 1989; 30: 400–404.

22. Cook JD, Skikne BS, Bayne RD. The use of the serum transferrin receptor for the assessment of iron status. In: Hallberg L, Asp NG, eds. Iron, Nutrition in Health and Disease. London: John Libbey, 1994: 49–58.

23. Bloem MW, Wedel M, Egger RJ, Speek AJ, Schrijver J, Saowakontha S, Schreurs WH. Iron metabolism and vitamin A deficiency in children in northeast Thailand. Am J Clin Nutr 1989; 50: 332–338.

24. Allen LH. Vitamin B_{12} metabolism and status during pregnancy, lactation and infancy. In: Allen L, King J, Lönnerdal B, eds. Nutrient Regulation During Pregnancy: Lactation and Infant Growth. New York: Plenum Press, 1994: 173–186.

25. Cordingley FT, Crawford GP. Giardia infection causes vitamin B_{12} deficiency. Aust NZ J Med 1986; 16: 78–79.

26. Swanson CA, King JC. Zinc and pregnancy outcome. Am J Clin Nutr 1987; 46: 763–771.

27. Fenton V, Cavill I, Fisher J. Iron stores in pregnancy. Br J Haematol 1977; 37: 145–149.

28. Huddle J-M, Gibson RS, Cullinan TR. Is zinc a limiting nutrient in the diets of rural pregnant women. Br J Nutr 1998; 79: 257–265.

29. Kirksey A, Wachs TD, Yunis F, Srinath U, Rahmanifar A, McCabe GP, Galal OM, Harrison GG, Jerome NW. Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. Am J Clin Nutr 1994; 60: 782–792.

30. Knight EM, Spurlock BG, Edwards CH, Johnson AA, Oyemade UJ, Cole OJ, West WL, Manning M, James H, Laryea H, Westney OE, Jones S, Westney LS. Biochemical profile of African American women during three trimesters of pregnancy and at delivery. J Nutr 1994; 124: 943S–953S.

31. Goldenberg RL, Tamura T, Neggers Y, Johnston KE, DuBard MB, Hauth JC. The effect of zinc supplementation on pregnancy outcome. JAMA 1995; 274: 463–468.

32. Tamura T, Goldenberg RL, Johnston KE, DuBard M. Maternal plasma zinc concentrations and pregnancy outcome. Am J Clin Nutr 2000; 71: 109–113.

33. Osendarp SJ, van Raaij JM, Arifeen SE, Wahed MA, Baqui AH, Fuchs GJ. A randomized, placebo-controlled trial of the effect of zinc supplementation during pregnancy on pregnancy outcome in Bangladeshi urban poor. Am J Clin Nutr 2000; 71: 114–119.

34. Hambidge KM, Krebs NF, Jacobs MA, Favier A, Guyette L, Ilke DN. Zinc nutritional status during pregnancy: a longitudinal study. Am J Clin Nutr 1983; 37: 429–442.

35. Jameson S. Zinc status in pregnancy: the effect of zinc therapy on perinatal mortality, prematurity, and placental ablation. Ann NY Acad Sci 1993; 678: 178–192.

36. WHO. Pregnant and lactating women. Physical Status: The Use and Interpretation of Anthropometry. Geneva: WHO, 1995: 37–120.

37. Institute of Medicine. Nutrition during pregnancy, weight gain and nutrient supplements. Report of the Subcommittee on dietary intake and nutrient supplements during pregnancy. Subcommittee on Dietary Intake and Nutrient Supplements During Pregnancy, Committee on Nutritional Status During Pregnancy and Lactation, Food and Nutrition Board. Washington DC: National Academy Press, 1990: 1–233.