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Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) study

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Summary. A prospective cohort study with a 1-year follow-up of 156 neonates was carried out specifically designed to test the hypothesis that there is a positive relationship between iron deficiency during pregnancy and the development of the same disease in newborn infants. Exposure was defined as being born of a mother with ferropenic anaemia at delivery, and cases as the infants who developed iron deficiency during their first year of life. A statistically significant positive association was detected with an odds ratio of 6.57 (95% confidence limits 1.81–25.97). A stratified analysis was also performed to control the effect of potential confounders such as socio-economic variables, feeding practices and other factors linked with the iron status of infants. This second analytical procedure showed no alteration in the association detected in the simple analysis but that there was a statistically significant strong interaction between the quantity of cow's milk intake and the ferropenic status of the mother. These results show a relationship between iron deficiency of the mother at delivery and the development of iron deficiency in the infants. These new findings could be important in the development of new prevention programmes applied to pregnant women.

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Introduction

Iron deficiency is still a major public health problem in developed countries. This condition is particularly prevalent in childhood, leading to important consequences in children's well-being, behaviour and learning processes.

Many epidemiological studies have been carried out to determine the associated risk factors, and harmful feeding practices have been identified.[1–3] Even when these have been eliminated, however, the incidence of iron deficiency is unacceptably high. Consequently it is important to identify other risk factors, including those which can occur during pregnancy, because of their relevance to the development of preventive measures.

Some authors,[4–7] and a cross-sectional study performed in the Spanish region of Valencia,[8] support the idea that maternal iron deficiency during pregnancy is a risk factor for infant iron deficiency. This conflicts with the more prevalent idea[9–15] that the iron status of the newborn is relatively independent of the mother's iron status during pregnancy. This paper presents the results of the Valencia Infant Anaemia Cohort study (VIAC), a project designed specifically to test this hypothesis by following infants throughout their first year of life.

Material and methods

Study design

The VIAC is a prospective cohort study comparing the incidence of iron deficiency in two neonatal cohorts during a 1-year period. The exposed cohort was made up of newborns whose mothers had ferropenic anaemia at the moment of delivery, and the unexposed cohort consisted of randomly selected newborns from mothers without iron deficiency.

Sample characteristics

We used the results of a previous cross-sectional study,[8] to calculate the minimum sample size needed to test the hypothesis.[16] The expected frequency of iron deficiency among the non-exposed group was estimated as 10%, the expected relative risk was 5, and the significance level was established at 5% with a power of 90%. The total sample size required was established as 30 exposed and 30 unexposed neonates.

During the study period the Spanish public health services covered about 90% of the population, those not covered by these services being the very rich and the very poor. The former seek care in the private sector and the latter in charity hospitals; thus our sample is composed mainly of middle-class urban women.

The study was carried out in the University Hospital of Valencia owned by the national public health service, which has a catchment area of around 200 000

inhabitants. The exposed cohort comprised all the anaemic women who delivered in the hospital from May 1985 to May 1986. The unexposed group was selected during the same period and comprised the next two women who delivered after an identified anaemic mother in the same hospital. All the women and newborn who presented special pathological conditions which could modify their haematological tests were excluded from the study. Only neonates from singleton pregnancies, with a gestational age of 38 weeks or more, birthweight of 2500 g or more, and without any evidence of neonatal diseases, chronic pathology during infancy or primary haematological diseases were included in the study. We performed the same laboratory tests in both exposed and unexposed groups.

Follow-up schedule

Both groups, exposed and unexposed, were monitored throughout 1 year. An active schedule of follow-up including mail and phone calls was developed. The iron status of the mothers was studied 24 hours after delivery, and the iron status of the infants was studied at birth, and subsequently at the age of 3,6,9 and 12 months.

The socio-economic data were obtained by personally interviewing the mothers within the first 36 hours after delivery. Clinical data referring to the pregnancy, delivery and neonatal period were obtained from the clinical records. At each follow-up visit, the mothers were also personally interviewed to find out the feeding habits of their infants and to establish whether there existed any condition such as iron therapy, infection or serious disease which could invalidate the control measurements.

Definition of exposure and cases

All the women included in the study and their infants were classified according to their serum ferritin, erythrocyte protoporphyrin and haemoglobin level, either as normal or in the three classic stages of iron deficiency:[17]

(1) Depletion of iron stores: serum ferritin <12 ng/ml.
(2) Ferropenic erythropoiesis: serum ferritin <12 ng/ml and erythocyte protoporphyrin >35 mcg/100 ml of whole blood.
(3) Ferropenic anaemia: haemoglobin below 11 g/dl and at least one of the conditions in stage 2.

A good response to iron therapy (1 g/dl in 2 months) was also required to classify infants as suffering from ferropenic anaemia. This was used to exclude those with any other type of anaemia.

Blood samples were obtained by finger puncture after warming the extremity to facilitate a free flow of blood and avoid any squeezing of the finger. Serum

ferritin determinations were performed using the ELISA technique (Ferrizyme TM, Abbott Laboratories), erythrocyte protoporphyrin was measured in a ZNP 4000 Hematofluorometer (Environmental Science Associates, Burlington MA, USA), and haemoglobin concentrations were determined by using the cyanmeta-haemoglobin technique (Drabkin reactive, QCA, Tarragona, Spain).

In order to improve the efficiency of the comparison *non exposure* was defined as being born from a mother without abnormalities on the haematological test. Neonates from mothers in stage 1 of iron deficiency were not enrolled in the study. We did not find any mothers with stage 2.

Exposure was defined as being born of a mother with stage 3 of iron deficiency and *cases* were defined as those infants that developed stage 2 or 3 iron deficiency during their first year of life. Infants with stage 1 (depletion of iron stores) were considered as non-cases because of the discussion about its pathological meaning. Non-ferropenic anaemia was excluded from the definition of exposure and cases.

Epidemiological analysis

Cumulative incidence was used as a measure of frequency; the odds ratio (OR) was used as an association measure, with precision indicated by confidence limits.[18] After the simple analysis, a stratified one was performed to detect confounding and interaction, using the Mantel-Haenszel summary estimator with Cornfield's 95% confidence limits and the chi-square for heterogeneity to detect interaction.[18]

The following variables were used in the analysis:

(1) Parents: age (cut-off point: 25 years), marital status, immigration, educational level (4 years of education), occupation and income (70 000 pesetas/month).
(2) Pregnancy: weight/height ratio of the mother, number of children, time interval between pregnancies (more or less than 2 years), blood loss or other condition during pregnancy, iron therapy and weight gain.
(3) Neonate: birthweight (more or less than 3 000 g), sex, breast feeding and its duration (more or less than 3 months), age at the introduction of fruit juices (before or after 4 months), solid foods (before or after 4 months), fruits (before or after 6 months), non-milk proteins (before or after 6 months), whole cow's milk (before or after 6 months) and daily intake of whole cow's milk (more or less than 500 ml).

The data were analysed on a Sperry Computer using BMDP.[19]

Results

Table 1 shows the distribution of the child's ferric status by that of the mother.

Table 1. Distribution of the child's ferric status by mother's status

Mother's ferric status	Child's ferric status				
	1	2	3	Normal	Total
Stage 3	13	6	8	20	47
Stage 1	26	1	2	14	43
Normal	23	2	2	39	66
Total	62	9	12	73	156

In all, 63 women were defined as exposed at delivery, i.e. with haemoglobin level between 8.7 and 11 g/dl. One refused to participate in the study, so the exposed cohort comprised 62 neonates. Of the 47 (76%) who completed the 1-year follow-up period, 13 (28%) developed stage 1, 14 (30%) developed a compromised erythropoiesis — 6 were stage 2 (13%) and 8 stage 3 (17%) — and 20 (42%) were of normal status.

The non-exposed cohort was composed of 90 women, all of whom initially agreed to participate. Of these, 66 (73.3%) completed the 1-year follow-up period: 23 (35%) developed stage 1 iron deficiency, 2 (3%) developed stage 2, 2 (3%) developed stage 3 and 39 (59%) had normal status (Table 1).

Table 2. Number of cases during the follow-up period according to the iron deficiency stage

Iron deficiency stage[17]	Month of follow-up						Total	
	6		9		12			
	2	3	2	3	2	3	2	3
Exposed	1	1	1	4	4	3	6	8
Unexposed	1	2	1	—	—	—	2	2

Table 2 shows the distribution of cases according to exposure status and month of diagnosis. The exposed group developed iron deficiency later (9–12 months) than the unexposed one (6–9 months). No iron deficiency was detected at 3 months. None of the infants had iron deficiency symptoms at the time of diagnosis.

Table 3 shows the results of the simple analysis of the data using as outcome variables the development of stages 2 or 3 iron deficiency. The odds ratio indicates

Table 3 Association between anaemic status in the mothers and stages 2 or 3 iron deficiency in their infants

		Infant	
		Anaemia	No anaemia
Mother	Anaemia	14	33
	No anaemia	4	62

OR=6.57; 95% Cornfield's CI=1.81–25.97; chi-square=11.44; P=0.0007.

OR=odds ratio; CI=95% confidence interval.

a positive association between the anaemic status of the mothers and iron status of their infants.

The results of the stratified analyses are shown in Table 4. No confounding was detected. Every summary estimator maintains the statistically significant association. In addition a statistically significant strong interaction was detected between the quantity of cow's milk ingestion and the ferropenic status of the mother in pregnancy.

Discussion

The idea that the fetus has complete priority when there is maternal deficiency of factors needed for erithropoiesis has been maintained by many authors. Following this rationale the neonate would be affected by his/her mother's iron deficiency only if the mother suffers from very severe anaemia during pregnancy.[9–12] Underlying this theory is the idea that preventive manoeuvres during pregnancy to treat iron deficiency in the mother have no important effect on the iron status of the child.

There has been considerable controversy in recent years concerning this theory. Some studies which have detected an association between mother's iron

Table 4. Stratified analyses

Variable	χ^2	Summary odds ratio	95% Confidence intervals
Mother			
Age	0.06 (P=0.81)	6.77	(1.86,27.43)
Marital status	1.91 (P=0.17)	6.28	(1.85,28.65)
Immigration	0.60 (P=0.44)	6.63	(1.78,25.85)
Educational level	1.82 (P=0.18)	6.31	(1.80,25.92)
Occupation	0.68 (P=0.41)	7.19	(1.89,28.04)
Weight/height	2.49 (P=0.29)	7.40	(1.75,41.67)
Number of children	2.12 (P=0.14)	5.59	(1.63,24.19)
Interpregnancy interval	0.09 (P=0.76)	6.14	(1.70,25.01)
Father			
Age	0.02 (P=0.87)	6.15	(1.65,24.68)
Educational level	1.10 (P=0.29)	6.08	(1.64,24.45)
Occupation	1.61 (P=0.20)	5.98	(1.54,23.18)
Family income	0.00 (P=0.96)	6.20	(1.69,24.74)
Pregnancy			
Blood losses	0.78 (P=0.38)	7.73	(1.96,31.84)
Diseases	0.39 (P=0.53)	6.36	(1.76,28.31)
Iron therapy	3.12 (P=0.08)	4.09	(1.24,21.51)
Weight gain	2.79 (P=0.25)	4.74	(1.23,20.21)
Neonatal disease	0.42 (P=0.51)	6.89	(1.86,29.85)
Birthweight	1.33 (P=0.25)	6.79	(1.84,26.90)
Sex	0.03 (P=0.85)	6.57	(1.81,25.95)
Feeding			
Breast feeding	0.43 (P=0.51)	6.56	(1.80,28.35)
Duration	1.56 (P=0.46)	8.29	(2.02,32.74)
Juices	1.67 (P=0.43)	5.84	(1.66,24.82)
Solid food	1.34 (P=0.25)	6.63	(1.80,27.05)
Fruit	1.19 (P=0.27)	6.50	(1.88,28.19)
Non-milk proteins	0.78 (P=0.67)	8.20	(2.73,28.49)
Whole cow's milk	3.39 (P=0.07)	7.35	(1.88,28.65)
Whole cow's milk intake	7.97 (P=0.00)	4.37	(1.23,21.80)

χ^2=chi squared for heterogeneity.

deficiency status and the frequency of the same problems in their infants have been criticised on the grounds of their retrospective or cross-sectional nature.[7,20]

The VIAC project was specifically designed to test this theory using a prospective cohort design to avoid the problems of retrospective data and the lack of a follow-up period in the cross-sectional approach. A major concern in designing the study was to be able to measure the association between the

mothers' iron status and that of their infant independently of other risk factors such as socio-economic level or feeding practices.

Our study could be criticised because we have found a higher frequency of iron deficiency among the exposed group due to the influence of variables associated with the exposure as well as with the disease (e.g. low birthweight, feeding practices, etc). The results of the stratified analysis in Table 4 are conclusive in this respect, no confounding was detected and the association found could be considered as independently attributable to the biological effect of the mother's iron status on that of the infant.

We do not think the loss of 15 neonates from the exposed cohort and 24 from the non-exposed one an important source of bias. The rate of losses in the follow-up period was not excessively high and was quite similar in both groups (24% exposed *vs* 27% non-exposed). In any case, if this bias existed then our relative risk would most likely be biased towards unity because losses to follow-up in paediatric clinics are in general associated with variables such as a low socio-economic level which are associated with an increased risk of anaemia in both mothers and infants.

As far as we know, this is the first 1-year follow-up cohort study to test the relationship between mother and infant iron deficiency, and it contradicts the accepted dogma of the relative independence of mother and infant iron deficiency. It supports the hypothesis of a relationship between the newborn's low iron status and the mother's iron deficiency during pregnancy.[6] Nevertheless, further studies are needed in order to quantify the relationship in comparison with those of the other risk factors associated with infant iron deficiency (low socio-economic level, incorrect feeding practices, etc). Although other studies are needed to further clarify the pathogenic mechanisms and the possible influence of confounding variables using multivariate analysis, we think that we have enough evidence to establish a new rationale in the development of preventive measures for iron deficiency in infants by controlling iron deficiency in pregnant women.

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