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Prematurity and maternal folate deficiency: Anemia during pregnancy study group results in Valencia,Venezuela

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SUMMARY. The purpose of this study was to determine the association and its magnitude between prematurity and folate deficiency in women in their third trimester of pregnancy, and at labor. An incident case - control study was conducted using 2 controls per case. Data was obtained in a tertiary hospital in Valencia, Venezuela. A total of 543 women who delivered between May and December 1996 entered into the study. Women having a preterm delivery (<37 weeks of gestation at delivery) were defined as cases (n = 181). Anemia was defined according to WHO as Hb less than 11g/dL, when a pregnant woman had a folate serum level < 3 µg/ml was considered a folate deficiency. Logistic regression was used to analyze the data and likelihood ratio test was done for model comparison. Folate deficiency was found to be significantly associated with prematurity (Odds Ratio: 1.97; 95%CI = 1.06 to 3.68 *P* = .032), after adjusting for prior preterm labor, prenatal care visits, prior abortion, prior fetal death, placental abruption, and premature rupture oval membranes. In conclusion, maternal folate deficiency at the end of the third trimester of pregnancy, at labor, was associated with an increased risk of prematurity.
Key words: Prematurity, folate deficiency, odds ratio, case control, risk, pregnancy, third trimester, Venezuela.

RESUMEN. Prematuridad y deficiencia de ácido fólico: Resultados del grupo de estudio de anemia materna en Valencia, Venezuela. El objetivo del trabajo fue determinar la asociación y su magnitud entre prematuridad y deficiencia de folato. Se utilizó un diseño de casos y control (2 controles por caso), realizado en la Maternidad "Dr. J.L. Facchín de Boni", (Valencia, Venezuela). Entre mayo y diciembre de 1996 fueron estudiadas 543 embarazadas al final del tercer trimestre gestacional y en trabajo de parto. Los casos (<37 semanas de gestación, OMS) fueron 181. La anemia fue definida como la presencia de Hb < de 11g/dL, según la OMS. La deficiencia de folato fue definida como folato sérico < 3 µg/ml. Los datos fueron analizados mediante regresión logística. Para evaluar la significancia de los modelos reducidos se utilizó la prueba de razón verosimilitud. Se determinó que la prematuridad está asociada significativamente con deficiencia de folato (OR: 1.97 IC95% = 1.06 a 3.68, *P* = .032), después de ajustar por desprendimiento placentario, ruptura prematura de membranas, partos prematuros previos, historia de abortos, historia de muerte fetal y menos de 5 visitas prenatales. En Valencia, Venezuela, la deficiencia de ácido fólico está asociada con un mayor riesgo de prematuridad.
Palabras clave: Prematuridad, deficiencia de folatos, odds ratio, casos y control, riesgo, embarazo, tercer trimestre, Venezuela, ácido fólico.

INTRODUCTION

Anemia is the main hematological complication during pregnancy. According to the World Health Organization (WHO) (1), the diagnosis of anemia during pregnancy is established when the hemoglobin (Hb) level is below 11 g/dL, being this the borderline between "physiologic anemia during pregnancy" and true anemia during pregnancy. All over the world, anemia during pregnancy is a public health problem (2,3). The nutritional anemia is the most important cause of maternal anemia. Folate deficiency (FD) is considered as the second cause of nutritional anemia.

Two reports (4,5), suggest that low folic acid intake increases the risk for preterm delivery. However, Mahomed (6) based on a systematic review in the Cochrane Library of Systematic Review suggests that there is not enough evidence to evaluate whether folate supplementation has any effect, beneficial or harmful, on clinical outcomes for mother and baby. On the other hand, the effect of supplementing the diet with folic acid given preconceptionally or in the first half of pregnancy was a decrease in the incidence of preterm labor (7). So, there is a controversy on the impact of maternal folate deficiency (FD) on pregnancy outcome.

All studies about this issue, with or without association between prematurity and folic acid, have been performed in developed countries. The importance of this study is due to the fact of having been carried out in a developing country, in a particular city (Valencia, Venezuela) where there is a prevalence of 12% of FD during pregnancy (8).

The main objective of the present research is to determine

the association between prematurity and maternal FD, at the end of third trimester of pregnancy. A case-control study with incident cases was performed.

METHODS

Subjects and data acquisition

The "Valencia Anemia during Pregnancy Study -VAPS-" is a large study about prevalence of maternal anemia during third trimester, carried out between May and December of 1996. It was done in Maternidad "Dr. J.L. Facchín de Boni" of Ciudad Hospitalaria "Dr. Enrique Tejera," in Valencia, Venezuela. Details of the methods are given in other paper (9). Briefly, both cases and controls came from the VAPS above mentioned. Since pregnant women entered at labor, as much the cases as the controls delivered the same day from the admission to hospital. The inclusion criteria for this study were stated as all pregnant women in their third trimester of gestation at labor. Women with multifetal pregnancies and pregnant women who did not remember their last menstrual period (LMP) were excluded.

A medical history and physical examination were performed for all patients. A questionnaire was used to obtain information about sociodemographic, obstetric, medical non-obstetric, drugs, and exposure to toxic substance data. Gestation age at delivery was determined by last menstrual period and was confirmed by clinical examination in each patient. In each newborn, Capurro's test was done.

By using WHO criteria (1), maternal anemia was defined as Hb less than 11 g/dL in any stage of gestation, and prematurity (10) was defined as any delivery of a live single infant between 24 and 36 weeks of gestation. Only serum folate was measured in this research. We used a Folate Radioaasay Kit [^{125}I] (ICN Pharmaceuticals, Orangeburg, N.Y, USA). FD was defined when serum folate level was lower than 3 μg/ml according to Wagner (11).

Any pregnant woman having systolic blood pressure higher than or equal to 135 mm Hg and/or diastolic blood pressure higher than or equal to 85 mm Hg, or receiving antihypertensive therapy at admission was considered as hypertensive patient. Smoking and alcohol during pregnancy were also recorded. Uterine bleeding was categorized without, bleeding in just one trimester and with bleeding in more than two trimesters.

No attempt was made to match the controls for age, parity, or any other variable. Ethnic group classification was not attempted due to the considerable race mixture in our population.

At labor, 12 ml of venous blood were obtained in a EDTA containing tube to perform a complete blood count (CBC) using an electronic counter Cobas Helios 3ä (Roche Diagnostic Systems). Blood sample was analyzed at main hospital laboratory within a 2-hour period after drawn.

This research was approved by the Ethical Committee of the Ciudad Hospitalaria "Dr. Enrique Tejera", and free informed consent was obtained from all patients enrolled into the study.

Data analysis

Sample size was determined assuming 37% prevalence of anemia in the controls, with an expected minimum prevalence of 50% in cases. In addition, it is assumed a 95% confidence level, 80% power, and a ratio of 2 controls per case. The final sample size was 543 pregnant women (181 cases and 362 controls). Epi Info software (version 5.0, CDC, Atlanta, Ga) was used to perform sample size calculation.

Data are presented as mean±SD unless otherwise noted. Categorical variables were compared by using chi-square test or Fisher's exact test when it was appropriate. Continuous variables were analyzed by using the Student's *t*-test for unpaired data. Stratified analysis was done using the Mantel-Haenszel procedure. To adjust for potential confounding factors, multiple logistic regression was used in order to determine the association between prematurity and maternal anemia and its magnitude. Model evaluation was done by using likelihood ratio test (12-14). Only biologic variables and other variables clearly associated with prematurity were included in the initial model to avoid a final model that could lack logical explanation (15). Initial model was composed by prematurity as the dependent variable, maternal folate deficiency as the exposure variable, and age, obstetric variables [placental abruption, premature rupture of membranes (PROM), previous preterm birth, number of prenatal care visits (PCV) and uterine bleeding], hypertension and smoking, as potential confounders.

A two-tailed P value of less than 0.05 was considered to indicate statistical significance. Stata version 6.0 (Stata Corp, College Station, Houston, Tx) was used for statistical analysis.

RESULTS

In total sample, mean of serum folate levels (±SD) (n = 543) was 7.6(±5.5) mg% (95%CI = 7.1 to 8.0). Range was 0.4 to 24 mg%.

Mean age (±SD) in the non-FD group (n = 494) was 24.5(±6.7) years (95%CI = 23.6 to 24.8) while in the FD group (n=49) it was 23.3(±6.7) years (95%CI = 21.4 to 25.2 P = .37).

Mean of gestational age (±SD) in the non-FD group (n = 494) was 37.4(±3) weeks (95%CI = 37.1 to 37.7) while in the FD group (n = 49) it was 35.8(±3.4) weeks (95%CI = 34.9 to 36.8 P = .0007).

Mean of Hb (±SD) in the preterm group (n=181) was

10.71(±1.7) g/dL (95%CI = 10.46 to 10.96) while in the control group (n=362) it was 11.54(±1.4) g/dL (95%CI = 11.39 to 11.69 $P = .001$).

Of the five hundred and forty three pregnant women, 20.8% (113/543) did not receive folic acid supplement. In those patients (n =113), 55% had anemia.

Table 1 shows the risk of prematurity according to the serum folate's level of the pregnant women.

TABLE 1
Risk of prematurity in pregnant women with FD according to serum folate levels

Prematurity	Level of Serum Folate (µg/ml)					
	<=3.4	<= 3.0	<= 2.5	<=2	<=1.5	<=1
n	132	123	81	61	36	20
OR	2.71	2.81	3.6	4.4	3.97	5.4
95%CI	1.3 to 5.6	1.3 to 5.9	1.4 to 9.3	1.5 to 13.3	1.01 to 15.4	0.85 to 3.4
p	0.007	0.006	0.006	0.006	0.046	0.07

Crude evaluation of association between prematurity and FD showed an OR of 2.25 (95%CI = 1.25 to 4.05 $P = .005$). After adjustment for potential confounders, OR and their 95%CIs show that maternal FD during pregnancy in third trimester is an important predictor of prematurity. Table 2 shows the final model.

TABLE 2
Final model of association between prematurity and maternal folate deficiency

Variable	Odds ratio	95% CI	P
Folate deficiency anemia	1.97	1.06 to 3.68	.03
Placental abruption	12.7	1.47 to 109	.02
PROM	2.1	1.37 to 3.23	.001
Previous preterm delivery	3.0	1.53 to 5.92	.001
Prior abortion	1.5	1.06 to 2.01	.02
Previous fetal death	3.3	1.24 to 8.80	.01
Prenatal care visits			
No visits*	1		
1 to 4	0.9	0.51 to 1.76	0.87
5 to 7	0.6	0.33 to 1.07	0.08
³8	0.4	0.20 to 0.80	0.01

PROM: premature rupture of membranes
* reference group

DISCUSSION

Prematurity is the major cause of perinatal mortality (16). The findings of this study support our hypothesis that folic acid deficiency (FD) during pregnancy, evaluated during third trimester, and at labor, is a risk factor for prematurity.

After adjustment for potential confounding factors, we have shown that the effect associated with FD remains recognizable. Therefore, our results suggest that there is an increased risk of a poor obstetric outcome when the level of serum folate is less than 3 µg/ml. Our results have concordance with what Sifakis and Pharmakides (17) stated about the fact that FD is more common in women who are not receiving prenatal folic acid supplements.

What mechanisms could explain the association between FD and prematurity? Folic acid plays an important role in the conversion of homocysteine in methionine (18). The relationship between serum folate and homocysteine may be useful for detecting borderline folic acid deficiency in pregnancy (19). A metabolic effect of folic acid deficiency is an elevation of blood homocysteine, so, total homocysteine (tHcy) measured in serum or plasma is a marker of folate status. Therefore, the biological plausibility could be explained using the possibility of occurrence of hyperhomocysteinemia. Epidemiological studies have shown that increased serum homocysteine concentrations well inversely correlated with folate concentrations (20). Increased circulating total homocysteine concentrations are associated with higher risk for premature vascular disease (21,22). Since, hyperhomocysteinemia disturbs the vascularization of the placenta and thereby reduces its function, a hypothesized pathway is a gene-environment interaction based on a highly prevalent mutation in the gene for methylenetetrahydrofolate (MTHFR), combined with low folic acid intake, consequent hyperhomocysteinemia, and decidual vasculopathy (23). In Hordaland Homocysteine study, Vollset et al (24) have pointed out that elevated tHcy concentration is associated with common pregnancy complications and adverse pregnancy outcomes.

Hyperhomocysteinemia could be mediated, at least, by two mechanisms: nutritional depletion of folic acid (by low intake and/or by increased catabolism) and/or as an indirect consequence of endogenous overuse of antioxidant vitamins (folic acid) during prolonged states of immune activation (25). Due to increased needs for fetal growth, placenta, and maternal tissues, pregnancy imposes stress on folate stores (26), it seems to be due to the accelerated breakdown of the vitamin because of its participation in cellular biosynthesis. According to studies in rat models, an increased folate turnover may occur during pregnancy, it means elevated rates of folate catabolism (27). Higgins et al (28) estimated rate of folate catabolism in pregnant and non-pregnant women and they found that rate progressively increases during pregnancy reaching a peak in the third trimester at the time of maximal fetal growth. McPartlin et al, measuring folate breakdown products p-amino-benzoylglutamate (pABGlu) and its acetylated derivate p-acetamidobenzoylglutamate (apABGlu), have also found accelerated folate breakdown in pregnancy (29). Therefore, the hypothesis could now be

formulated as: a high homocysteine level in the blood, even with normal folic acid levels, could be the toxic agent for the developing embryo.

During pregnancy, *what should the daily supply of folic acid be*? During pregnancy, a daily intake of 400µg has been advised, and it has been argued that synthetic folic acid (tablets or enriched food) is much better absorbed and more readily available than natural folic acid (30). However, based on the results of a study on the relationship between increased folate catabolism and the increased requirement for folate in pregnancy, Higgins et al (28) have recommended that dietary allowance for folic acid during pregnancy should be 430 µg in the second trimester and 540 µg in third trimester. Besides, Cuadill et al (31) suggest that 450 µg/day is sufficient to maintain folate status in pregnant women. In brief, the folic acid supplementation doses fluctuate between 400 and 540 µg/day. Folic acid is a micronutrient of particular importance for prevention of adverse pregnancy outcomes (32,33).

We believe this study may have two limitations; interpretation of the results must take these into account. First, there is a chance of recall bias in the process of gathering data. Given low income and low socioeconomic status of the pregnant women of this study, it was not feasible to carry out longitudinal studies. These studies tend to be more costly and need many logistic problems in their execution. Second, it is difficult to determine the prevalence of folic acid deficiency in the pregnant women because of the criteria used to define folic acid deficiency, even though we used the usually accepted criterion (serum folate level <3.0 µg/ml). There are large inter- and intra- methods variations, estimated dietary folic acid intakes are not reliable, and we determined only serum folate concentrations. However, in our country this is the first time that a research like this has been carried out.

In conclusion, according with these results, there is a risk of prematurity in pregnant women with folic acid deficiency, therefore, it is advisable the consumption of this micronutrient during the pregnancy. Folic acid is key for optimal macronutrient metabolism because of its essential role in metabolism. Undoubtedly, preterm delivery has a multifactor origin where it is not discarded a chronic degenerative process in the placenta intimately associated with a folic acid imbalance. The need to design an educational program about appropriate use of prenatal care may be one of the implications of this study. Educational efforts addressing appropriate use of prenatal care should be initiated in our city. All efforts to change patterns of use of the prenatal care program must be encouraged.

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