European Journal of Clinical Nutrition (2007) 61, 382–386
 0954-3007/07 $30.00
www.nature.com/ejcn

ORIGINAL ARTICLE

Folate, vitamin B12 and total homocysteine levels in neonates from Brazil

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Objective: To determine folates, vitamin B12 and total homocysteine levels among neonates from mothers of low or high socioeconomic status.
Design: We carried out a cross-sectional transversal study comprising 143 neonates from two maternity hospitals in the city of Salvador, Northeast of Brazil. Cord blood samples were obtained at the time of delivery from newborns from low (group 1, $n=77$) or high (group 2, $n=66$) socioeconomic status. The vitamin B12 and folates were analyzed by electrochemiluminescence immunoassay and by a competitive test using a natural folate-binding protein (FBP), respectively. Total homocyteine levels were measured by fluorescence polarization immunoassay. Maternal environmental risk factors for pregnancy complications were obtained from all mothers.
Results: Only 2% of women from group 1 received prenatal care/vitamin supplementation, whereas almost all mothers from group 2 (96%) were properly followed. Anemia and/or infections pre- or during pregnancy was more prevalent among mothers of babies from group 1. Folate levels among newborns from group 1 and 2 were 7.38 ± 2.71 and 8.83 ± 4.06 ng/ml, respectively. No difference in the vitamin B12 levels was determined between groups. In addition, tHcy serum levels were higher among newborns from group 1 compared to those from group 2 (8.54 ± 4.06 vs 6.35 ± 1.33 μmol/l, respectively; $P=0.005$).
Conclusion: These results demonstrate that unprivileged young woman has limited accesses to prenatal care, present high-risk factors that hamper both maternal and newborn health. Maternal and newborn health status could be improved by simply reinforcing the use of folate-enriched diet. The work presented illustrates the challenges that developing countries have to face in order to provide preventive adequate health care to the population at large.
European Journal of Clinical Nutrition (2007) **61,** 382–386. doi:10.1038/sj.ejcn.1602528; published online 20 September 2006

Keywords: newborns; homocysteine; vitamin B12; folate; pregnancy; socioeconomic status

Introduction

Pregnancy is a period of rapid growth and cell differentiation for both mother and fetus (McArdle and Ashworth, 1999). The demand for nutrients increase during pregnancy (Ladipo, 2000) and approximately 20% of women show deficiency of vitamin B12 and/or folate (Bruinse and van den Berg, 1995; Pardo *et al*., 2000; Mathews *et al*., 2004). Several environmental risk factors such as poor nutritional status, parasitosis and lifestyle may conduct to serious health problems in pre- or pregnant women. Epidemiological and biological evidence suggest that acute- or chronic-specific nutritional deficiencies can contribute to severe maternal and fetal morbidity and mortality (Nieuwenhuis, 2003). In most developing countries, nutritional deficiencies of both macro- and micronutrients are common in women of reproductive age (Villar *et al*., 1983; Romero *et al*., 1989; Barker, 1995; Goldenberg and Andrews, 1996; McGaw, 2002). Serum or plasma level of the nutrients can be influenced by cigarette or alcohol drugs. Cigarette smoking during pregnancy is known to be harmful and can result in increased spontaneous abortions in the first trimester, premature placenta abruption, preterm delivery, decreased birth weight and sudden infant death syndrome (Lambers and Clarke, 1996). Maternal alcohol consumption during pregnancy may lead to fetal growth retardation, malformations, developmental defects and/or spontaneous abortion (Thomson, 1978; Kumar, 1982).

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Received 1 February 2005; revised 3 July 2006; accepted 3 August 2006; published online 20 September 2006

Periconceptional administration of multivitamin and/or folic acid supplementation, prevent recurrent neural tubes defects (Smithells *et al*., 1989; Czeizel and Dudás, 1992), megaloblastic anemia and neurologic alterations (Pippard, 1994; Campbell, 1995). Vitamin B12 and folate are important cofactors involved in homocysteine metabolism (Girelli *et al*., 1998). The high serum or plasma levels of total homocysteine (tHcy) has been received considerable interest because it has been associated with high risk for renal disease, neurodevelopmental processes and cardiovascular disease (Anderson *et al*., 2004; Fowler, 2005; Picker and Coyle, 2005; Verhoef and de Groot, 2005). Therefore, the effect of chronic hyperhomocysteine may hamper to the health of both young and adults population, supporting the idea that early detection of vitamins deficiencies with specific vitamin supplementation offers the opportunity to prevent most of these inadvertent complications (Graham and O'Callaghan, 2000; Minet *et al*., 2000).

Brazil is a large country with one of the highest heterogeneous population owing to several waves of immigration, which resulted in cultural, socioeconomic and ethnic diversity among different geographic regions. Salvador is the largest city in Bahia, a Northeastern Brazilian state, with a population of 2.7 million people (RIIS, 1997) where 86% of the local population is of African origin (Azevedo *et al*., 1981).

Few studies have been described nutritional standard levels of macro- and micronutrients among Brazilian mothers and newborns. The current study aimed to compare folate, vitamin B12 and tHcy levels among groups of newborns from two maternity hospitals that provide assistance to subjects of high or low socioeconomic status.

Materials and methods

Subjects

The study was approved by the Oswaldo Cruz Research Foundation's human research ethics committee. Cord blood samples were carried out only after signed informed consent form was obtained. We carried out a cross-sectional transversal study comprising 143 newborn babies randomly selected from a group of 843 neonates, as reported (Couto *et al*., 2004). The babies were born from February to December 2000 in two different maternity hospitals, the Tsylla Balbino, a public maternity hospital and the Santo Amaro, a private maternity hospital located in city of Salvador. These two maternity hospitals provide care for large number of pregnant women of low (Tsylla Balbino Hospital; group 1) or high (Santo Amaro Hospital; group 2) socioeconomic status.

Group I included 77 newborns randomly chosen of a total of 675 (11.4%) during the study period. All 77 samples were tested for vitamin B12 and folic acid levels and 35 of 77 for tHcy levels. Group 2 consisted of 66 newborns randomly chosen of a total of 276 (26.4%), in which all were analyzed for vitamin B12 and folate and 40 of 66 had tHcy levels determined. These groups consisted of apparently healthy newborns of mothers with uncomplicated pregnancy, without known systemic disease or illicit drug usage. All clinically relevant information required for both groups were obtained by one of the authors and included current or former history of cigarette smoking, diabetes mellitus (repeated fasting plasma glucose levels higher than 6.7 mmol/l), systemic arterial hypertension (diastolic blood pressure higher than 90 mm Hg or use of anti-hypertensive drug), non occasional alcohol consumption (more than once a week) and infectious diseases.

Blood collection

Cord blood samples were collected using sterile tubes without anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, USA). Samples were refrigerated at 4°C for a maximum of 8 h; serum and red blood cell pellet were separated and kept frozen until the analysis.

Biochemical analysis

The folate and vitamin B12 serum levels were measured by electrochemiluminescence immunoassay (ECLIA) (Roche Laboratories, Mannheim, Germany), and by a competitive test using a natural folate-binding protein (FBP) (Roche Laboratories, Mannheim, Germany), respectively, according to manufacturer's instructions. The tHcy serum levels were measure by fluorescence polarization immunoassay (Abbott Laboratories, Oslo, Norway).

Statistical analysis

EPI Info Software version 6.04 (16) was used as data bank and parametric variables were compared by Student's *t*-test, nonparametric variables by the Wilcoxon rank sum test. The Software SPSS version 10 was utilized for analysis of linear regression. The tHcy serum level was considered as a dependent variable and folate and vitamin B12 serum levels were the independent variables. The model utilized for analysis of linear regression was controlled for the two independent variables. A *P*-value of less than 0.05 was considered statistically significant.

Results

Mothers from public hospital care present high prevalence of environmental risk factors for poor nutritional status

The clinical and laboratory data of mothers who gave birth to babies from both groups are shown in Table 1. The results clearly demonstrate differences that characterize the study population in terms of access to adequate health care and importantly preventive therapies. The most important difference was regarding the assisted prenatal care, which

was regular for only few (2%) of women from low social economic background whereas almost all mothers from group 2 were properly followed. Regarding alcohol consumption, mothers in group 1 tend to have a larger consumption compared to mothers in group 2, although the difference between both groups was not statistically significant. These data reinforce the selection's criteria used in the study by selecting subjects from distinct hospital that provide assistance to the population based on their socioeconomical status.

Newborns from mothers of low socioeconomic status presented lower folate levels, but not vitamin B12 levels, than those born from high socioeconomic status

Cord blood samples were tested for both folate and vitamin B12 in the same laboratory and the results are summarized in Table 2. The mean levels of folate for newborns from group 1 was lower than those levels determined among babies from group 2 (7.38 vs 8.83 ng/ml, respectively; $P=0.01$). However, no differences in vitamin B12 levels were detected between newborns from both groups.

Although we have not determined the normal levels of folate and vitamin B12 in cord blood samples from subjects of the general population, the folate and vitamin B12 values showed here are in agreement with reports in other Brazilian populations (Guerra-Shinohara *et al*., 2002; Alessio *et al*., 2004).

Table 1 Clinical data and environmental risk factors pre and during gestation

	Socioeconomic status		*Statistical analysis* (P)
	Low (group 1) n = *77(%)*	*High (group 2)* n = *66 (%)*	
Age, years (mean ± s.d.)	23.1 ± 5.9	23.4 ± 4.2	NS
First time pregnancy	34 (44)	31 (47)	NS
Regular prenatal care	3 (2)	65 (92)	<0.0001
Current/former smoker	11 (19)	5 (7.5)	NS
Alcohol consumption	23 (31)	12 (18)	NS
Anemia	49 (64)	17 (26)	<0.0001
Urinary infection	19 (25)	4 (5.5)	0.026

Abbreviations: NS, non-statistical significant differences; s.d., standard deviation.

Homocysteine plasma levels are higher among newborns of mothers of low socioeconomic status when compared to those of high socioeconomic status

We next determined whether tHcy levels in cord blood samples obtained from babies from both groups differed. The results shown in Table 2 represent data obtained in approximately half of the newborn tested for both folate and vitamin B12 levels. The data demonstrate that tHcy mean levels were higher among newborns from group I than those from group 2 (6.35 vs 8.54 μmol/l, respectively; $P=0.01$). The linear regression analysis shows a negative correlation between folate and tHcy serum levels, $r=-0.278$, $P=0.01$ (Figure 1) but not statistically significant association when comparing vitamin B12 and tHcy serum values ($r=-0.190$, $P=0.09$) (Figure 2).

Discussion

According to World Health Organization (WHO) guidelines, about 11 million of children, aged 0–4 years, are dying every year worldwide, with a frequency of 99% in developing

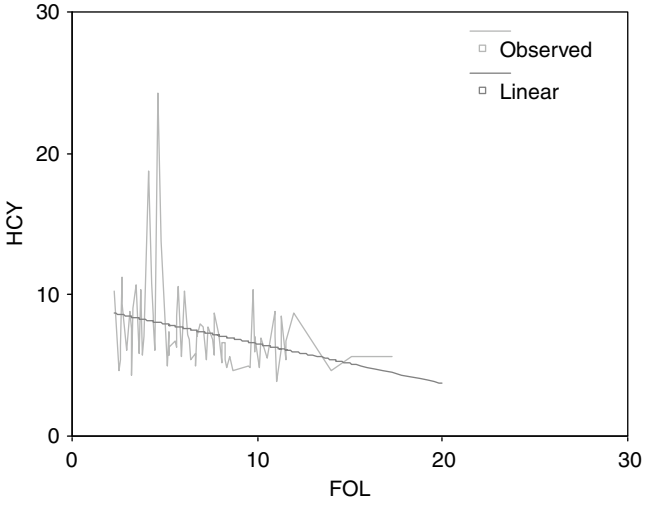

Figure 1 Linear regression analysis of folate (ng/ml) and tHcy (μmol/l) serum levels in the total of 75 newborns from both public and private maternity hospitals, $r=-0.278$, $P=0.01$.

Table 2 Cord blood micronutrient levels of newborns according to social economic status

Socioeconomic status	*Cord blood folate, vitamin B12 and total homocysteine levels mean values (± s.d.)*		
	Folate (ng/ml)	*Vitamin B12 (pg/ml)*	*Total homocysteine (μmol/l)*
All newborns (*n*)	7.80 ± 3.51 (143)	579.30 ± 245.98 (143)	7.40 ± 3.1 (75)
Low Group 1(*n*)	7.38 ± 2.71 (77)	586.07 ± 243.97 (77)	8.54 ± 4.06 (35)
High Group 2 (*n*)	8.83 ± 4.06 (66)	572.81 ± 250.62 (66)	6.35 ± 1.33 (40)
Statistical[a] analysis (P)	0.01	0.75	0.005

Abbreviation: s.d., standard deviation.
[a]Wilcoxon test.

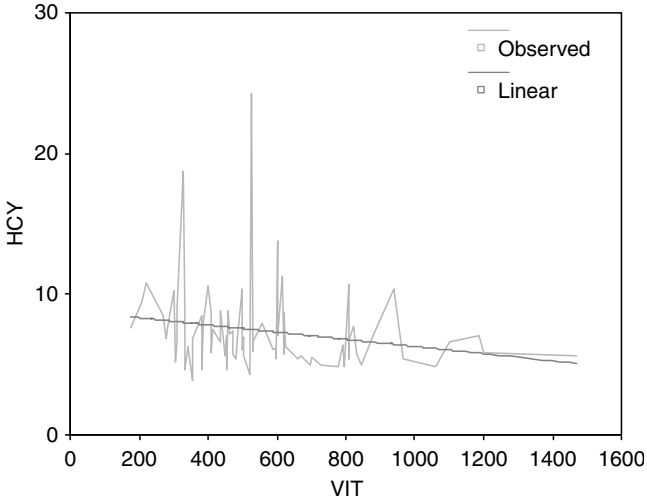

Figure 2 Linear regression analysis of vitamin B12 (pg/ml) and tHcy (μmol/l) serum levels in the total of 75 newborns from both public and private maternity hospitals, $r = -0.190$, $P = 0.09$.

countries. Malnutrition is associated with more than 60% of these deaths among those very young (WHO, 2002). Guerra-Shinohara *et al* (2002) suggested that deficient pregnant women are unable to provide the necessary amounts of several micronutrient including vitamin B12 and folate to their fetuses. This study described the newborn nutritional status by measuring vitamin B12, folate and tHcy levels in babies born from two distinct maternity hospitals that provide assistance to low or high socioeconomic status people. Although the maternal nutritional status was not investigated, the results from the babies are naturally an approximation of these vitamins and tHcy levels in the mothers and most probably reflect the maternal nutritional status. The data demonstrated that unprivileged young woman has limited accesses to prenatal care, present high-risk factors that hamper both maternal and newborn health. One important point was the higher number of mothers, among the low socioeconomic status group, using cigarette and alcohol. Though these trends were not significant, these factors have been demonstrated to be harmful to pregnancy (Thomson, 1978; Kumar, 1982; Lambers and Clarke, 1996), needing further investigation in this population.

The consequences of the inadequate prenatal care found in this study could be appreciated by further evidence on the differences of micronutrients, notably folate levels, among newborns from low socioeconomic status when compared to those of higher social status. These findings are of surprising because, folate is abundant in local diet, a geographic area that is rich in tropical fruits and vegetable, but may not be sufficient when chronic malnourished women become pregnant. In contrast, vitamin B12 levels between newborns from both groups were within the normal range. Vitamin B12 deficiency is most often a result of impaired gastrointestinal absorption, such as in the case of pernicious anemia. Dietary deficiency of vitamin B12 is very unusual and it occurs mainly in vegetarians who also avoid dairy products, such as eggs or in people under severe general malnutrition. As these factors were not common in the target population of the current study, severe vitamin B12 deficiency should not be a common finding. In addition, there are some limitations in the laboratory assessment of vitamin B12 deficiency. Moderate serum vitamin B12 levels (100–400 ng/ml) overlap with measurements in normal individuals but may be associated with severe vitamin B12 deficiency and only those levels and <100 ng/ml are highly predictable of the severe deficiency (William *et al*., 1995).

The folate and vitamin B12 serum levels are important cofactors involved in homocysteine metabolism (Girelli *et al*., 1998; Minet *et al*., 2000). The tHcy levels have been considered to be a sensitive functional marker of these deficiencies (Ueland *et al*., 1993). Here we showed that tHcy levels are higher among newborns of mother from low socioeconomic status when compared to those born from healthier mothers; in addition, there was a negative correlation between tHcy and folate. The same correlation was found in recent report among normal Brazilian children (from 1 to 8 years old) from Southeast Brazil (Alessio *et al*., 2004). The transient hypermocyteinaemia and mild hypertriglyceridemia have been associated to an inflammatory activation of the endothelium (Mansoor *et al*., 2004). An hyperhomocysteinaemia has also been described to increase thrombotic risk in hypertensive patients by increase platelet activation and decrease soluble *P*-selectin (Spencer *et al*., 2004). Although there are important results in these researches, the role of homocysteine as a casual factor or as a marker for coronary heart disease, needs to be explained. The high tHcy serum levels are relatively easy to correct by the use of folic acid at low doses, such as 0.5 mg/day (Oakley *et al*., 1996), which has been proven cost effective. There is an epidemiological evidence that folic acid supplementation and fortification might protect against the increased risk of neural tube defects, vascular disease and some types of cancer (Bailey *et al*., 2003; Johnson, 2004; Green and Miller, 2005). Moreover, Verhoef and de Groot (2005) showed that folic acid could be more effective than dietary folate in lowering tHcy concentration.

This work presented illustrates the challenges faced by developing countries to provide adequate health care. Although the numbers of the subjects enrolled in this study are relatively modest, the data obtained are sufficient to indicate further guidelines to improve both maternal and newborn health by simply reinforcing the use of enriched diet. In addition, it is also important to increase the awareness about environmental risk factors that are important contributors in hampering the maternal–fetal health. The socioeconomic diversity between the groups studied here was a critical factor in determining deficient nutritional status. This study's findings probably could be the basis for the Brazilian healthy public program aimed to improve the health of unprivileged populations by talking simple

preventive rather than therapeutic and often more expensive strategies.

Acknowledgements

We are grateful to all the subjects who participated in this study and for the nurses and physicians who provided the cord blood samples. We thank Lucio Borba from the Roche Laboratory (Salvador-Bahia) for providing the folate and vitamin B12 kits. *Research Grant Support*: CNPq (306524/2004-0 to MSG), FAPESB (1431030005540 to MSG), Brazilian Department of Healthy (3111 to MSG).

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