CLINICAL RESEARCH

Erythrocyte Volume, Folate Levels, and the Presence of Methylenetetrahydrofolate Reductase Polymorphism

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Homozygosity for a common polymorphism in the 5,10 methylenetetrahydrofolate reductase (MTHFR) gene (C677T) has been associated to an increased risk of neural tube defects as well as derangements in folate, homocysteine, and hematological parameters. This study analyzed the relationship between folate levels, the erythrocyte volume, and the presence of homozygosity for the C677T polymorphism in a group of 126 Puerto Rican healthy women of childbearing age. Blood samples were analyzed for erythrocyte mean corpuscular volume (MCV), mean erythrocyte hemoglobin content (MCH), folate, and RBC folate. Homozygosity for the C677T mutation was determined by PCR. Thirty-two percent (32%) of women used a folic acid supplement during the three months prior to sampling. Mean folate and RBC folate levels were within the normal range. Individuals homozygous for the MTHFR C677T polymorphism had no elevation of MCV (p=0.70) or MCH (p=0.68). Women in the lower quartile of folate levels did not show differences in their MCV or MCH. In this sample of Puerto Rican women, homozygosity for the C677T MTHFR polymorphism was not associated to elevations of MCV or MCH even in the presence of lower folate levels.

Key Words: Erythrocyte volume, Folate, Folic acid, Methylenetetrahydrofolate, Puerto Rico, Neural tube defects

The thermolabile methylenetetrahydrofolate reductase (MTHFR) was initially described in patients with hyperhomocysteinemia(1). The enzyme 5, 10 MTHFR catalyzes the reduction of 5, 10 methyltetrahydrofolate, the predominant circulatory form of folates, carbon donor for the re-methylation of homocysteine to methionine(2). A mutation was described as a homozygous C to T transition in the 677 base of the MTHFR cDNA. The C677T mutation in 5,10-MTHFR and the decreased activity of this enzyme have been associated to higher plasma homocysteine levels and the development of vascular disease. It also causes a redistribution of folates, mainly the red cell folate, and lowered plasma folate(3).

Many studies have related the presence of the thermolabile enzyme with an increased risk of having a neural tube defect affected pregnancy(4). In Puerto Rico, as in other Hispanic populations, there is a high incidence of neural tube defects. Folic acid supplementation is being used as a strategy to prevent these congenital malformations. Supplementation may overcome mild defects in enzyme function, preventing elevations of homocysteine(5). More recently, the polymorphism has been associated to the development of structural congenital heart disease(6).

It has been suggested that individuals with the homozygous C677T MTHFR mutation have a disturbed folate metabolism and increased folate requirement. It is known that folate deficiency leads to macrocytic, hyperchromic anemia possibly due to disturbed DNA synthesis and methylation. An elevation of MCV and MCH in homozygous carriers of the C677T polymorphism with low folate levels has been reported(2). We studied the relationship between folate levels, the erythrocyte volume, and the presence of homozygosity for the C677T polymorphism in a group of Puerto Rican women of childbearing age.

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Materials and Methods

A group of 126 healthy Puerto Rican women employed at the Puerto Rico Medical Center were included in the study. The protocol was approved by the Institutional Review Board. Exclusion criteria included family history of neural tube defects, malabsorption states, and pregnancy. Blood samples were taken for a complete blood count, folate, red blood cell (RBC) folate, and the C677T variant.

The prevalence of the C677T mutation was examined by PCR. Three ml of blood were collected from each individual in EDTA tubes. DNA was isolated by standard laboratory procedures. DNA was amplified by polymerase chain reaction (PCR) followed by allele-specific restriction enzyme digestion with HinfI as described by Frosst et al(7). Individuals were genotyped TT if homozygous for the C677T thermolabile allele, CT if heterozygous, and CC if no mutation was identified.

Chi-square and analysis of variance were used to test interactions between genotypes and haematological parameters. A p-value less than 0.05 was considered significant.

Results

Mean age of women was 34 years (range 22-57). Thirty-two percent (32%) of them used a folic acid supplement during the three months prior to sampling. The use of folic acid was associated to higher levels of folate (p=0.0038), and RBC folate (p=0.0001). Median folate and RBC folate levels were within the normal range as well as mean MCV and MCH . Homozygosity for the C677T was observed in 8% of the subjects. Homozygous subjects (TT) were not different from normal (CC) and heterozygous (CT) individuals in folate, RBC folate, MCV, and MCH. Individuals in the lower quartile of folate levels (<10.7 ng/dl) were not different from those with higher levels with respect to MCV (p=0.2133) and MCH (p=0.2431). The same was true for RBC folate (<301 ng/dl). Table 1 shows the haematological parameters according to MTHFR genotype.

Table 1. Haematological Parameters by MTHFR Genotype

	Genotype			
	CC n=49	CT n=62	TT n=10	p-value
Folate (ng/dl)	14.35 (7.6-53)	14.7 (6.1-73.5)	19.7 (9.5-49.2)	0.0966
RBC folate (ng/ml)	374 (211-991)	382 (204-991)	548 (272-954)	0.2883
MCV (fL)	87.9 (76.4-96.8)	87.3 (74.1-93.9)	88.2 (74.0-94.3)	0.7026
MCH (pg)	29.9(24.7-33.9)	29.7 (24.2-33.5)	30.2 (25.4-32.8)	0.6810

Discussion

Our studies represent the first investigation of folic acid metabolism in the Puerto Rican female population in childbearing age. Only 32 percent of the women were using a folic acid supplement despite a national campaign for its use which has been underway since 1994. Women who reported its use had higher levels of folate and RBC folate, as expected. The prevalence of the 5,10 MTHFR polymorphism was 8% in the study group. It is similar to the 5-15% reported in other populations(8). A higher prevalence has been reported in Mexicans(9). As a group, the studied females presented normal values of folate and RBC folate. Although derangements in folate and RBC folate have been reported in the literature in homozygotes for the 5, 10 MTHFR mutation, we did not find a significant difference between groups. However, the number of homozygotes in this sample was very small.

A report by Lalouscheck(2) showed that the 5, 10 MTHFR mutation exerts a strong influence on the relation between folate status and specific haematological parameters, particularly the MCV. Homozygous carriers with low folate levels showed an elevation in MCV and MCH. In our sample, no difference was found in MCV and MCH even in the presence of low folate levels (< 25th percentile).

The findings of this study have to be confirmed in a larger and more representative sample of the Puerto Rican population. However, it serves as a pilot study in the goal to understand the high incidence of neural tube defects in Puerto Rico. Analysis of homocysteine levels will help us understand better the interactions between the 5, 10 MTHFR genotype and haematological parameters.

Resumen

El estado homozigótico para un polimorfismo común en el gen de la metilenotetrahidrofolato reductasa(C677T) se ha asociado a un riesgo aumentado de defectos de tubo neural y a alteración en los niveles de folato, homocisteína y otros factores hematológicos. Este estudio analizó la relación entre niveles de folato, el volumen de eritrocitos y el ser homozigótico para el polimorfismo C677T en un grupo de 126 mujeres puertorriqueñas saludables y en edad reproductiva. Las muestras de sangre fueron analizadas para volumen corpuscular promedio (MCV), contenido de hemoglobina promedio en los eritrocitos (MCH), folato y folato en las células rojas. El estado homozigótico para la mutación C677T fue determinado por reacción

de polimerasa en cadena. Treinta y dos porciento (32%) de las mujeres usaron un suplemento de ácido fólico durante los tres meses previos a la toma de muestras. El folato promedio y los niveles de folato en las células rojas estuvieron dentro de los límites normales. Individuos homozigóticos para el polimorfismo C677T no presentaron elevación de volumen corpuscular promedio(p=0.70) o del contenido de hemoglobina promedio en los eritrocitos (p=0.68). Las mujeres con niveles más bajos de folato no presentaron diferencia en su volumen corpuscular promedio o en el contenido de hemoglobina promedio en los eritrocitos. En esta muestra de mujeres puertorriqueñas, el ser homozigótico para el polimorfismo C677T no se asoció a elevación del volumen corpuscular promedio o del contenido de hemoglobina promedio en los eritrocitos aún ante niveles bajos de folato.

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