

Total Homocysteine Levels in Healthy Children from the Monterrey Metropolitan Area, Mexico

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Abstract: Currently, there are indications for determining hyperhomocysteinemia in adulthood as risk factors for cardiovascular diseases, psychiatric disorders, pregnancy complications, birth defects, cognitive impairment in the elderly, in addition to cancer. If hyperhomocysteinemia is determined from childhood, it may be modulated with the provision of an opportunity for public health intervention. The objective of this descriptive study was to determine total homocysteine (tHcy) levels in healthy children from the Monterrey metropolitan area in Mexico. In a peripheral-blood sample collected from 56 healthy children aged 2–10 years, we determined tHcy concentration by high performance liquid chromatography (HPLC) with fluorescence detection. The geometric mean \pm SD was $9.78 \pm 1.73 \mu\text{mol/l}$. tHcys of the children studied were homogeneous by age cohort and gender. Nutritional state was classified by body mass index (BMI). Sixty five percent of children who participated in the study had normal BMI, and 96% of the children belong to the low socioeconomic status. In conclusion, to our knowledge this is the first-ever information on homocysteine (Hcy) prevalence in

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a population of healthy Mexican children. tHcy concentration was higher than that reported in other populations studies. This preliminary study could constitute the baseline for future public health studies.

Introduction

Homocysteine (Hcy) is an amino acid produced during the metabolism of methionine into cysteine. Several factors – both modifiable and non-modifiable – interact with Hcy metabolism and determine its concentrations. These include nutritional status, lifestyle, disease, age, gender, or ethnicity, respectively (Asnani et al., 2002).

Currently, there are indications for determining hyperhomocysteinemia in adulthood as a risk factor for cardiovascular disease, psychiatric disorders, pregnancy complications, birth defects, cognitive impairment in the elderly, in addition to cancer (Wu and Wu, 2002; Refsum et al., 2004). If hyperhomocysteinemia is determined from childhood, it may be modulated by lifestyle (diet), providing an opportunity for public health intervention (Greenlund et al., 1999; Bates et al., 2002). Nevertheless, there are few populations with Hcy determinants in samples of healthy children.

Studies of total Hcy (tHcy), comprising all forms of thiol derivatives that make up homocysteine by reduction with the high performance liquid chromatography (HPLC) method in clinically normal children, which have included the US and several Western and Eastern European countries, report geometric means values of 4.3–6.21 $\mu\text{mol/l}$ (Tostand et al., 1996; De Laet et al., 1999; Greenlund et al., 1999; Bates et al., 2002; Must et al., 2003).

Reports for Latin-American populations are few and employ other methods of measuring tHcy and/or do not express results as the geometric mean (Rogers et al., 2003; Aléssio et al., 2004). The objective of this descriptive study was to determine tHcy levels in healthy children (grouped by gender and three age cohorts) from the Monterrey Metropolitan area, in Mexico.

Material and Methods

A sample of 56 unrelated healthy children aged 2–10 years (Vilaseca et al., 1997), who attended the medical unit accompanied by their parents (none of the cases were patients). This medical unit was the Zone 33 Regional Hospital of the Instituto Mexicano del Seguro Social (IMSS), which receives patients from the metropolitan area of Monterrey, Nuevo León State, Mexico.

All parents signed an informed consent letter and the project was approved by the IMSS Ethics Committee. On a survey sheet, we registered the child's place of birth, height, and weight, as well as the parents' birthplaces and occupations. Recorded weight and height of all children included in the study were carried out by a trained nutrition staff member. We determined body mass index (BMI) by means of Centers for Disease Control (CDC) growth charts, 2000.

A 5-ml sample of peripheral blood was taken in the morning before the child ate breakfast and with the child in a seated position. This peripheral blood was collected in heparin-containing tubes and centrifuged at $1000 \times g$ for 10 min as soon as possible (<30 min). Finally, the plasma was stored at -20°C until analysis.

Determination of tHcy concentration was performed by HPLC by fluorescence detection utilizing an HPLC system and AccQtag instruction manual (Millipore corp., Massachusetts, USA) and the method for fluorescence detection (250-nm excitation wavelength, 395-nm emission wavelength), and gradient elution (Waters Corp., Milford, MA, USA) following the method cited by Hernanz et al. (1999) and Fermo et al. (1992) with slight modifications, employing DL-homocysteine (Fluka 53510) as internal control. In each run, we analyzed three concentrations that were different from the internal control (DL-homocysteine + control plasma). We confirmed that the inter-assay CV for the tHcy in control samples was less than 10% in all cases (Refsum et al., 2004).

Statistical analysis

All questionnaires were entered into a data base for statistical analyses, which were conducted in five parts using SPSS software. First, a statistical description of the plasma tHcy dataset was examined, and a positively skewed co-efficient (skewness co-efficient, 5.1) was found. Second, because tHcy was not normally distributed, we utilized \log_e , followed by back-transformation where appropriate for geometric mean. Third, transformed data were analyzed by the Levene test for variance. And fourth, one-way ANOVA with Newmann-Keull's test for multiple comparisons at the interpopulational level was employed to investigate any possible difference between tHcy levels and BMI in children grouped by age cohort (cohort I: 2–3.99 years of age; II: 4–6.99 years of age, III: 7–9.99 years of age) and gender (boys and girls). Fifth, Student *t*-test was utilized to compare tHcy levels and BMI between boys and girls. A P-value of <0.05 was considered significant.

Results

In this study, 96% of children belonged to the low socioeconomic status (paternal activity was blue collar), and 65% had normal nutrition according to BMI.

Distribution of geometric means of Hcy values ranged from 8.05–11.51 $\mu\text{mol/l}$ (mean 9.78 $\mu\text{mol/l}$). Table 1 presents the distribution of tHcy and BMI in children studied by gender and age cohort. When Student *t*-test was applied, no significant differences were found in gender-evaluated characteristics, with the exception of cohort III for BMI ($t=2.39$; $P=0.036$). On the other hand, one-way ANOVA test was applied in all three age cohorts; only BMI showed a significant difference ($\mu\text{I} = \mu\text{II} \neq \mu\text{III}$) ($F=3.72$; $P=0.031$). When nutritional state was classified by BMI, 65% of children had normal weight, 18% had overweight, 10% had a risk for overweight, and 8% of children had low weight.

Discussion

tHcy increases throughout the lifetime – in old age it is approximately twice higher than of childhood. In addition, after puberty, males have higher concentrations than females, but this is similar at adult age (Jacques et al., 1999; Must et al., 2003). Hcy of children studied in the present study was homogeneous in all age and gender cohorts.

Intermethod differences for measuring plasma tHcy, emphasize the need for standardization (Ubbink et al., 1999). Results of Hcy levels in Mexican children were higher than in other populations of children measured by same HPLC method with transformation for geometric mean. Tonstand et al. (1996) in Norway studied 678 children aged 8–12 years with a geometric mean of 5.25 $\mu\text{mol/l}$. De Laet et al. (1999) in Belgium conducted a study with 178 children aged 5–9 years with a geometric mean of 6.21 $\mu\text{mol/l}$, while Bates et al. (2002) in the UK studied 51 children (age range 4–6.99 years) and 131 children (age range 7–10.99 years) with geometric means of 5.16 and 5.59 $\mu\text{mol/l}$, respectively. Greenlund et al. (1999) in the US studied 343 children with ages ranging from 5–8 years and found a geometric mean of 5.7 $\mu\text{mol/l}$. Must et al. (2003) in the US studied children aged 4–5 years; the study population was non-Hispanic Caucasian ($n=73$), non-Hispanic African-American ($n=99$), and Mexican-American ($n=105$), with reported geometric means of 4.4, 4.7, and 4.3 $\mu\text{mol/l}$, respectively. In Latin American, few studies have been carried out in children; our values in arithmetic mean (11.12 $\mu\text{mol/l}$) were higher than those of previous reports. In Brazil, Aléssio et al. (2004) studied 63 children with ages ranging from 1–8 years and found a arithmetic

Table1 – Distribution of total homocysteine (tHcy) and body mass index (BMI) in Mexican healthy children by gender and age cohort

Characteristic	Gender	Age cohort			
		I $\bar{X} \pm \text{SD}$ (n)	II $\bar{X} \pm \text{SD}$ (n)	III $\bar{X} \pm \text{SD}$ (n)	total $\bar{X} \pm \text{SD}$ (n)
^a tHcy ($\mu\text{mol/l}$)	boy	9.78 \pm 1.92 (12)	11.02 \pm 1.60 (18)	7.46 \pm 1.60 (8)	9.58 \pm 1.75 (18)
	girl	11.94 \pm 2.03 (6)	7.17 \pm 1.63 (7)	11.13 \pm 1.42 (5)	9.78 \pm 1.72 (38)
	total	10.49 \pm 1.92 (18)	9.78 \pm 1.67 (25)	8.67 \pm 1.60 (13)	9.78 \pm 1.70 (56)
^b BMI (percentile)	boy	16.38 \pm 2.40 (12)	16.72 \pm 3.40 (16)	17.11 \pm 3.70 (8)	17.74 \pm 3.85 (17)
	girl	16.35 \pm 1.28 (6)	15.24 \pm 1.10 (6)	22.39 \pm 4.03 (5)	16.69 \pm 3.15 (36)
	total	16.37 \pm 2.05 (18)	16.31 \pm 3.07 (22)	19.14 \pm 4.56 (13)	17.03 \pm 3.39 (53)

^ageometric media, age cohorts: I = 2–3.99 years, II = 4–6.99 years, III = 7–9.99 years; ^b($\mu\text{I} = \mu\text{II} \neq \mu\text{III}$) $F=3.72$, $P=0.031$

mean of $8.65 \mu\text{mol/l}$. Rogers et al. (2003) in Guatemala conducted a study with 180 children 8–12 years of age, with a arithmetic mean of $9.24 \mu\text{mol/l}$; 9% were $>12.0 \mu\text{mol/l}$.

Nutritional and genetic factors must be considered in tHcy-level evaluation. Nevertheless, according to expert consensus or clinical experience, the common cause for increased tHcy in children comprises folate or cobalamin (vitamin B12). This cobalamin functions as a cofactor for methionine synthase and L-methylmalonyl-CoA mutase, and methionine synthase catalyzes the conversion of homocysteine to methionine (Refsum et al., 2004). There is a strong parallelism with respect to relationships between tHcy and vitamin-B status indices at all ages (Bates et al., 2002), and data from Mexico exhibited the same trend (Montaño-Loza et al., 2004). In addition, in Mexico this is considered prevalent, with severe vitamin B12 deficiency (Stabler and Allen, 2004). It is very probable that our studied population has a greater prevalence of these deficiencies than previously studied populations, and that this is the cause of differences of our tHcy levels. Thus, it would be highly appreciated whether vitamin replacement therapy could help in an ameliorating homocysteine levels in our population. Other important factor is child obesity; BMI of 18% of population studied was above 85th percentile, obesity in children and adolescents has been associated with an increased risk of low vitamin B12 concentration (Pinhas-Hamiel et al., 2006).

In Mexican adults, a study reports values higher than deficiencies in other populations; a case-control study on coronary atherosclerosis (study population aged 60.8 ± 12.6 years) showed a high prevalence of hyperhomocysteinemia ($>12 \mu\text{mol/l}$), this is significantly different from previous populations and who were not associated with evidence of coronary atherosclerosis (Montaño-Loza et al., 2004).

There are data demonstrating ethnic differences in genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR), an important regulatory enzyme in Hcy and folate metabolism (Refsum et al., 2004). No random convenience samples were investigated by Esfahani et al. (2003) in 433 unrelated women, and Mexican women (18.1%) had a higher frequency of the MTHFR 677TT (hyperhomocysteinemia-associated) genotype compared with Caucasian (7.2%), Asian (3.8%), and African-American (0%) women; additionally, a tendency was demonstrated for Mexican women with the 677TT genotype to have lower red cell folate concentrations than those with another polymorphism (677CC).

The high prevalence of this genotype may acquire greater meaning (i.e. affectation of the phenotype) in Mexico, a country without a consolidated program of folic acid fortification in staple food items. Additional long studies in Mexico of different socioeconomic status are necessary and will complement our result with genetic determinants, such as the MTHFR 677C→T polymorphism and in blood, such as folate acid and cobalamin in a child population.

In conclusion, to our knowledge this report provides the first-ever information on the prevalence of homocysteine in a population of Mexican children. tHcy concentration was higher than that in studies of populations from the US, Europe and Latin America. This preliminary study may constitute the baseline for future studies of public health.

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