Journal of Medical Sciences

ISSN 1682-4474
Homocysteine: An Indicator of Methylation Pathway Alteration in Down Syndrome Children and its Regulation by Folic Acid Therapy

Hala D. El-Gindi and Hala M. Hussien

The purpose of this research was to evaluate the total homocysteine (t-Hcy) metabolism in Down Syndrome (DS) children and to determine whether the supplementation with folic acid therapy would shift the genetically induced metabolic imbalance. Thirty-five infants with DS (17 male and 18 females), their mean age 17.66±12.24 months were included in this study. They were selected among those attending the Genetic Out Patients Clinic, Children Hospital. Present results revealed that Down syndrome’s children had a significant increase of serum folic acid after treatment with folic acid therapy (18.91±3.59 vs 11.95±1.55 ng mL^{-1}), while no significant change in vitamin B_{12} (323.17±38.42 vs 358.36±57.43 pg mL^{-1}). There was a significant decrease in plasma t-Hcy level after treatment with folic acid therapy (11.79±0.92 vs 14.41±4.93 μmol L^{-1}). A significant negative correlation was found between t-Hcy and folic acid serum levels (r = -0.112; p<0.05). The present study concluded that, the regulation of methylation pathways in Down syndrome becomes important in light of possible normalization of the metabolic imbalance and the detection of increased sensitivity to therapeutic interventions.

Key words: Down syndrome, homocysteine, folic acid therapy, metabolic imbalance
INTRODUCTION

Down syndrome or trisomy 21, is a complex genetic disease resulting from the presence of 3 copies of chromosome 21. Some clinical features of patients with Down syndrome can be related to functional folate deficiency. These features include enhanced methotrexate sensitivity (Ueland et al., 1990), elevated mean corpuscular volume and gastrointestinal malabsorption (Pogribna et al., 2001). Impaired folate function may explain deoxymethionine-pool imbalance and elevations in folate sensitive fragile sites and DNA strand breaks. These are lesions that may be related to the high incidence of leukemia observed in patients with DS. Conceivably an impaired S-adenosylmethionine dependent transmethylation reaction may have a diversity of effects, including dysfunction of the central nervous system (Monsen and Ueland, 2003).

Fetuses and neonates are in a state of rapid cell turnover that requires a high rate of DNA synthesis. This high rate of DNA synthesis is associated with a great need for vitamin B₁₂, folate and vitamin B-6. Early detection of deficiencies is important, however, because the neurologic changes that take place after pronounced vitamin B₁₂ deficiency in infant may be irreversible. Megaloblastic anemia and neurologic disorders such as hypotonia and delay in psychomotor development in infants occur at later stage of deficiency and are evident only in severe cases of folate and vitamin B₁₂ deficiency (Minet et al., 2000). Serum vitamin concentrations have relatively poor sensitivity and specificity in detecting subjects with subtle changes suggestive of vitamin deficiency (Savage et al., 1994).

Metabolites, such as methylnalonic acid, total homocysteine (t-Hcy) and cystathionine, involved in enzymatic reactions dependent on vitamin B₁₂, folate and vitamin B-6 have been found to be sensitive estimates of both functional and intracellular deficiencies of these vitamins. Homocysteine especially is widely regarded as a reliable indicator for this purpose (Vilaseca et al., 1997).

The dual purpose of the present study was to evaluate the total homocysteine metabolism in DS children and to determine whether the supplementation with folic acid therapy would shift the genetically induced metabolic imbalance.

MATERIALS AND METHODS

Thirty-five infants with DS, (17 male and 18 females), their mean age 17.66±12.24 months were included in this study. They were selected among those attending the Genetic out Patients Clinic, Children Hospital, Cairo University. All patients were subjected to full history taking and clinical examination. All patients received a daily dose of folic acid equivalent to 5 mg (The Nile Co. For Pharmaceuticals and Chemical Industries, Cairo, A.R.E., Ree 115668, Made in Egypt) for one month. Plasma t-Hcy, serum folic acid and vitamin B₁₂, were estimated before and after treatment.

Methods: Venous blood samples were taken from every subject after an overnight fast; divided into two aliquots. The first aliquot was put into heparinized tubes. The second aliquot was left to clot. Plasma and serum were separated by centrifugation at 3500 rpm for 10 min and frozen at -20°C until analyzed. Plasma t-Hcy was estimated by competitive immunoassay (ELIA) method according to the manufacture instructions [Axis ‘Shield Assay, Axis-Homocysteine, Bieckeermundig 4, D-29614 Soltau, Germany]. Serum folic acid and vitamin B₁₂, were estimated by radio-immunoassay (RIA) method using kits obtained from Diagnostic Product Corporation (DRC).

Statistical analysis: SPSS for Windows Version 7.0 Computer Program was used for statistical analysis. All numeric data were expressed as mean±SE. Data were analyzed using a paired student t-test to compare means before and after treatment. Person’s correlation coefficient was used to determine the relationships between different values. For all tests a probability <0.05 was considered as significant.

RESULTS AND DISCUSSION

Our results revealed that Down syndrome’s children had mean value of serum folate (11.95±1.55 ng mL⁻¹) and vitamin B₁₂ (358.36±57.43 pg mL⁻¹), within the normal international values (Table 1).

Figure 1 shows a significantly increase of serum folic acid after treatment with folic acid therapy (18.91±3.59), (Fig. 1) while no significant change in vitamin B₁₂ (323.17±38.42) (Fig. 2). There was a significant decrease in plasma t-Hcy level after treatment with folic acid therapy (11.79±0.92) (Fig. 3).

In Fig. 4 significant negative correlation was found between t-Hcy and folic acid levels (r = -0.112; p<0.05) while there was a negative non significant correlation between t-Hcy and vitamin B₁₂ levels (r = -0.593; p>0.05) (Fig. 5).

Table 1: The biochemical parameters of children with down syndrome

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Folic acid (ng mL⁻¹)</th>
<th>t-Hcy (μmol L⁻¹)</th>
<th>Vit. B₁₂ (Pg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>11.95±1.55</td>
<td>14.41±4.93</td>
<td>358.36±57.43</td>
</tr>
<tr>
<td>Normal</td>
<td>7.32</td>
<td>2.15</td>
<td>175-800</td>
</tr>
<tr>
<td>values</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1: Serum folic acid level (Pg mL⁻¹) in children with down syndrome before and after folate therapy

Fig. 2: Serum vitamin B₁₂ (Pg mL⁻¹) level in children with down syndrome before and after folate therapy

Fig. 3: Plasma total homocysteine (μ mol L⁻¹) level in children with down syndrome before and after therapy

Fig. 4: Person’s correlation between plasma total homocysteine and folic acid

Fig. 5: Person’s correlation between plasma total homocysteine and vitamin B₁₂

Many micronutrients and vitamins are critical for DNA synthesis/repair and maintenance of DNA methylation patterns. Folate has been most extensively investigated in this regard because of its unique function as methyl donor for nucleotide synthesis and biological methylation. Deficiency of folate induces disruption of DNA as well as alterations in DNA methylation status (Fenech and Ferguson, 2001).

Folate and/or methyl group dietary supply provides the most compelling data for the interaction of nutrients and DNA methylation, because these dietary elements are directly involved in DNA methylation via one carbon metabolism. Not only dietary folate depletion decrease genomic DNA methylation in both human and animal models but a folate replete diet also may restore DNA methylation status (Rampersaud et al., 2000).

In this study, the serum folic acid, vitamin B₁₂, and plasma t-Hey levels were assessed to detect the alteration in methylation pathways in Down syndrome children. The mean values of serum folic acid and vitamin B₁₂ were within normal range as compared with the international normal range values at the beginning of the observation period.

These results also indicate an increase in mean value for plasma t-Hey level as indicator of deficiency of intracellular folate level. This observation agrees with...
Ueland et al. (2000) who reported that a mild hyperhomocysteinemia appear as an indicator of altered one carbon metabolism.

In spite of many studies showed low plasma level of t-Hcy in DS children with mean age 7.4 years (Chadeaux et al., 1985; Pogribna et al., 2001), they reported that low t-Hcy level was due to increase of cystathionine activity, so increase homocysteine degradation through the transsulfuration pathway. We may attribute our results to the young age of our sample and most of them are breast fed, which are cysteine-enriched diet resulting in a product inhibition of cystathionine-synthase (Yamamoto et al., 1995).

With regard to the results after therapy, we observed that there was a significant decrease in plasma t-Hcy level which may indicate deviation of methylation pathway. Serum folate level was significantly increased after therapy and comparing with plasma t-Hcy level, there was a negative significant correlation. This results may be in agreement with Pullin et al. (2001), who reported that there was a decrease in homocysteine concentration after supplementation of folic acid. Also serum folate increased significantly and serum vitamin B12 concentration remained unchanged. Friso et al. (2002), showed that genomic DNA methylation in peripheral blood mononuclear cells directly correlated with folate status and inversely correlate with plasma homocysteine levels.

The present results revealed that there was no change in serum vitamin B12 after therapy; but there was a negative non significant correlation between t-Hcy and vitamin B12. Wekramasimghe and Fida (1993), found that there was a significant and independent relation between homocysteine and B12 status assessed by holotranscobalamin concentration but not by total serum B12 and that is why the effects of vitamin B12 on homocysteine concentration are frequently masked by folate status. Quinnivan et al. (2002), found that after folate therapy, the inverse association between plasma homocysteine and serum vitamin B12 was strengthened.

CONCLUSIONS

The methylation might be important for the development of children with trisomy 21 after birth which is crucial for the function of myelin sheaths and for synthesis of neurotransmitters. So, the study of the regulation of methylation pathways in Down syndrome becomes important in light of possible normalization of the metabolic imbalance and the detection of increased sensitivity to therapeutic interventions. A daily dose of synthetic folic acid of 5 mg infants with Down syndrome decreases plasma levels of total homocysteine. The possible normalization one carbon metabolism (folate and homocysteine) may might ameliorate behavior and cognitive functioning.

REFERENCES


