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Short Communication

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*For further information about this article or if you need reprints, please contact:*

Hala M. Hussein
Department of Nutrition,
National Research Center,
Dokki, Cairo, Egypt

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# Homocysteine: An Indicator of Methylation Pathway Alternation in Down Syndrome Children and its Regulation by Folic Acid Therapy

[1]Hala D. El-Gindi and [2]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 μ mol $L^{-1}$). 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

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[1]Department of Health Peadiatric
[2]Department of Nutrition, National Research Center, Dokki, Cairo, Egypt

## 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 gastro intestinal malabsorpation (Pogribna *et al*., 2001). Impaired folate function may explain deoxynucleotide-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-adenosly methionine 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 $B_{12}$, folate and vitamin B-6. Early detection of deficiencies is important, however, because the neurologic changes that take place after pronounced vitamin $B_{12}$ deficiency in infant may be irreversible. Megalobastic 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 $B_{12}$ 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 methylmalonic acid, total homocysteine (t-Hcy) and cystathionine, involved in enzymatic reactions dependent on vitamin $B_{12}$, 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, Cario, A.R.E., Rcc 115668, Made in Egypt) for one month. Plasma t-Hcy, serum folic acid and vitamin $B_{12}$, 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 heparinize 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 (EIA) method according to the manufacture' instructions [Axis-Sheild Assay, Axis-Homocysteine, Bickbeerngrund 4, D-29614 Soltue, Germany]. Serum folic acid and vitamin $B_{12}$ 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 $B_{12}$ (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 $B_{12}$ (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 $B_{12}$ levels ($r = -0.593$; $p<0.05$) (Fig. 5).

Table 1: The biochemical parameters of children with down syndrome

| Parameters | Folic acid (ng $mL^{-1}$) | t-Hcy (μ mol $L^{-1}$) | Vit. $B_{12}$ (Pg $mL^{-1}$) |
|---|---|---|---|
| Patient | 11.95±1.55 | 14.41±4.93 | 358.36±57.43 |
| Normal values | 7-32 | 2-15 | 175-800 |

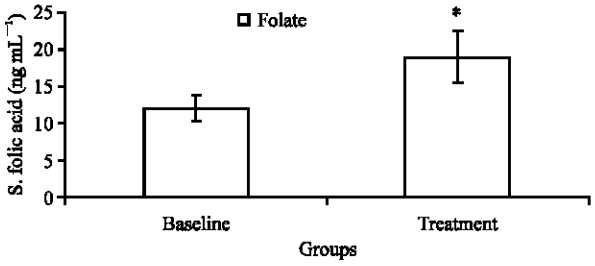


Fig. 1: Serum folic acid level (Pg $mL^{-1}$) in children with down syndrome before and after folate therapy

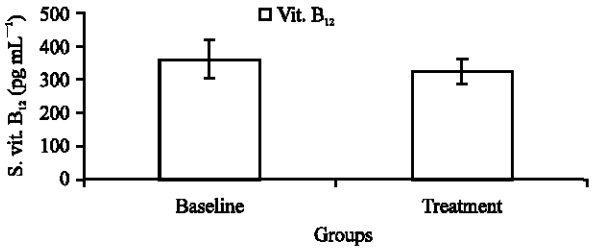


Fig. 2: Serum vitamin $B_{12}$ (Pg $mL^{-1}$) level in children with down syndrome before and after folate therapy

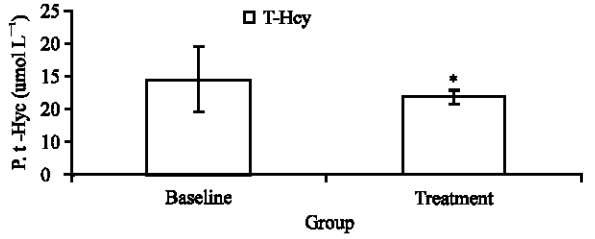


Fig. 3: Plasma total homocysteine (μ mol $L^{-1}$) level in children with down syndrome before and after therapy

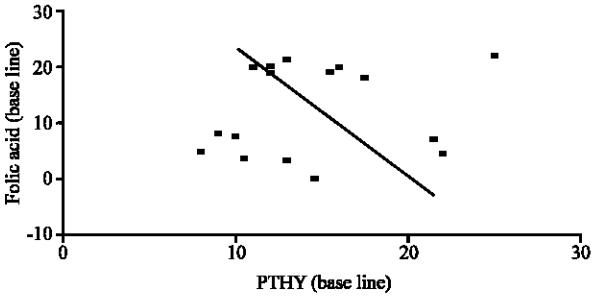


Fig. 4: Person's correlation between plasma total homocysteine and folic acid

Down's syndrome individuals exhibit significant disturbances in methylation pathways. The over expression of cystathionine bet- Synthase (located on the 21 chromosome) causes homocysteine to be converted into cysteine at an accelerated rate (Chadefaux, 1985).

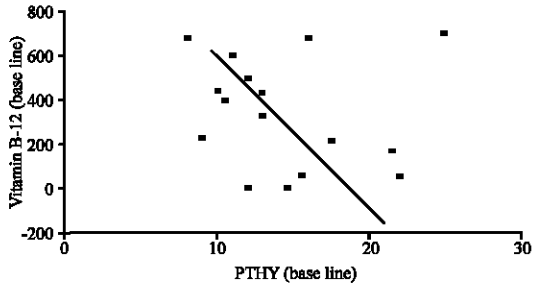


Fig. 5: Person's correlation between plasma total homocysteine and vitamin $B_{12}$

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 methyation. 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 $B_{12}$ and plasma t-Hcy levels were assessed to detect the alteration in methylation pathways in Down syndrome children. The mean values of serum folic acid and vitamin $B_{12}$ were within normal range as compared with the international normal range values at the beginning of the observation period.

This observation among serum folic acid and vitamin $B_{12}$ in our patients with Down syndrome agrees with the results of David *et al*. (1996), who found that erythrocyte and serum folates, vitamin $B_{12}$, serum iron and ferritin in DS children were not significantly different from those of control group, also they suggested that macrocytosis in DS children may not be an expression of reduced red cell survival but rather due to an alternate folate remethylation pathways. Also Gericke *et al*. (1977), reported that red cell folate value was very low in DS individuals although mean serum folate and vitamin $B_{12}$ levels were normal in this group.

These results also indicate an increase in mean value for plasma t-Hcy level as indicator of deficiency of intracellular folate level. This observation agrees with

Ueland *et al.* (2000) who reported that a mild hyper-homocytinemia 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 (Chadefaux *et al.*, 1985; Pogribna *et al.*, 2001), they reported that low t-Hcy level was due to increase of cystathionine activity, so increase homocystiene 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 cystiene-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 $B_{12}$ 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 homocystiene levels.

The present results revealed that there was no change in serum vitamin $B_{12}$ after therapy; but there was a negative non significant correlation between t-Hcy and vitamin $B_{12}$. Wickramasinghe and Fida (1993), found that there was a significant and independent relation between homocysteine and $B_{12}$ status assessed by holotranscobalamin concentration but not by total serum $B_{12}$ and that is why the effects of vitamin $B_{12}$ on homocysteine concentration are frequently masked by folate status. Quinlivan *et al.* (2002), found that after folate therapy, the inverse association between plasma homocysteine and serum vitamin $B_{12}$ was strengthed.

## 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 neurotransmiltors. 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.

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