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Folate and Homocysteine metabolism in Indian children with Down syndrome.

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Abstract

Down syndrome (DS) individuals present abnormalities in folate-homocysteine metabolism that is attributed to the additional copy of the Cystathionine β-synthase (CβS) gene located on chromosome 21. Therefore, the aim of the study was to estimate the plasma levels of folate and homocysteine (Hcy) and compare it with age and sex matched healthy controls. The results showed a significant decrease in Hcy levels in DS. Moreover, cases affected with congenital heart defects presented statistically significant increase in Hcy levels but the same was not observed with folate. Decreased levels of folic acid and homocysteine were observed in cases with neural tube defects but found insignificant. Decreased Hcy concentration leads to functional folate deficiency contributing to the metabolic pathology in DS. The study recommends folinic acid supplementation for DS individuals.

Keywords: Down syndrome, Oxidative stress, DNA damage, DNA hypomethylation, Folic acid, Homocysteine, Cystathionine β-synthase, MTHFR, Folinic acid, Congenital heart defects, Neural tube defects.

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Introduction

Down syndrome (OMIM-190685) or Trisomy 21 is the most common and one of the best known autosomal aneuploidy disorders among children. It is associated with more than 80 clinical traits including mental retardation, congenital heart diseases, neural tube defects and increased risk of childhood leukaemia [1].Oxidative stress induced DNA damage plays a major role in the clinical manifestations of Down syndrome (DS) due to over expression of Superoxide dismutase 1 (SOD 1), an antioxidant enzyme based in chromosome 21q 22.1 [2]. Folic acid functions primarily as one carbon unit donor involved in many important body processes including DNA/RNA synthesis and repair. Homocysteine (Hcy) is a non-protein-forming, sulphur amino acid situated in a critical regulatory branch point between trans-methylation and trans-sulphuration pathways (Figure 2) [3].It is evident that defective folate and homocysteine metabolism leads to DNA strand breaks, chromosomal instability, impaired DNA repair capability, DNA hypomethylation and abnormal gene expression [3, 4]. DS individuals present abnormalities in folate metabolism that is attributed to the additional copy of the Cystathionine β-synthase (CβS) gene located on chromosome 21 [5, 6]. Thus, overexpression of the CβS gene leads to an increase in the activity of trans-sulphuration pathway leading to reduction in the concentration of Hcy that is available for the remethylation reaction catalysed by methionine synthase. Simultaneously, the hyperactivity of the Hcy trans-sulfuration pathway leads to an accumulation of 5-methyltetrahydrofolate (5-MTHF) and a reduction in the conversion of 5-MTHF to Tetrahydrofolate (THF). THF is the metabolically active form of folate and is required for de novo synthesis of nucleotides required for DNA and RNA synthesis (Figure 2) [5]. Congenital heart defects (CHD) and Neural tube defects (NTD) have been associated with dietary folate deficiency and disturbed folate/homocysteine metabolism [7,8]. Therefore, the primary aim of the present study is to estimate the plasma levels of folic acid and homocysteine in DS cases and compare with that of normal controls. Also, investigate the role of folate/homocysteine metabolism in the etiology of CHD and NTD in DS

Material and Methods

Subjects in the current study consisted of 108 clinically diagnosed children with Down syndrome belonging to South Indian population. The mean ± SD age of the children was 3.03 ± 4.1 which included 64 males and 44 females. The control group was formed by 110 gender and age matched healthy normal children belonging to the same geographical area as cases. Based on age, cases were subdivided into five groups; Neonates (n=12), 1-12 months (n=29), 1-3 years (n=31), 3-5 years (n=18), above 5 years (n=18). Prior to the study, clearance was obtained

from Institute Human Ethics Committee, JIPMER hospital, Puducherry. Written consent was also obtained from the parents/guardians of both case and control group. All relevant clinical data regarding congenital anomalies involving cardiovascular and nervous system in cases was recorded. Peripheral venous blood was collected into a sterile tube with EDTA as anticoagulant under aseptic precautions by venipuncture. Clinical diagnosis of Down syndrome was followed by conventional lymphocyte cell culture for karyotyping and confirmation of the same by interphase-FISH using Olympus BX-51 epifluorescence microscope, Japan and automated karyotyping workstation, Ikaros/IsisMetasystem, Carl Zeiss-Germany [9].

Plasma levels of Folic acid and Homocysteine were estimated based on the principle of Competitive immunoassay employing AVIDA centaur folate assay kit and AVIDA centaur HCY assay kit respectively utilising the services of the auto analyser - ADVIA Centaur CP Immunoassay System, Siemens-Germany. Appropriate parametric (independent students t test) or non-parametric test (Mann Whitney U) was used for comparing the continuous data between the two groups. All statistical analysis was carried out at 5% level of significance and p value < 0.05 was considered significant. Analysis was done using SPSS (Version 19) software.

Results

Conventional lymphocyte cell culture and Interphase FISH confirmed the presence of pure trisomy 21 in all hundred and eight (108) clinically diagnosed children. Other variants of Down syndrome like partial trisomy, translocation and mosaicism were not observed. A total of 42 cases had CHD consisting of atrial/ventricular septal defect, Tetralogy/ Pentalogy of Fallot, patent ductus arteriosus etc and 18 cases had NTD. The results of Competitive immunoassay showed no significant difference in the values of folic acid between cases and controls. But, plasma levels of Homocysteine revealed 40% decrease in DS individuals compared to controls which was statistically significant (Table -1). At the level of individual age groups, folic acid was found to exhibit a gradual decrease in its levels with advancement in age and the level was conversely observed with Homocysteine (Figure 1). DS cases affected with congenital heart defects presented statistically significant increase in Homocysteine levels but the same was not observed with folate (Table 2). Decreased levels of folic acid and homocysteine were observed in cases with neural tube defects and they were statistically insignificant (Table 3).

Table 1. *Plasma levels of Folate and Homocysteine in DS cases and controls*

Groups	**Plasma levels - Mean ± Standard Deviation**	
	Folic acid (ng/ml)	**Homocysteine (µmol/L)**
DS Cases (n=108)	8.12 ± 2.42	5.66 ± 2.49
Healthy Controls (n=110)	8.48 ± 2.24	9.21 ± 4.24
p value	NS	**< 0.001**

NS – Not significant

Table 2. *Plasma levels of Folate and Homocysteine in DS cases with and without CHD*

Groups	**Congenital heart defects**		
	Present (n=42)	**Absent (n=66)**	**p value**
Folic acid (ng/ml)	7.99 ± 2.59	8.20 ± 2.33	NS
Homocysteine (µmol/L)	6.43 ± 2.73	5.17 ± 2.20	**0.009**

NS – Not significant

Table 3. *Plasma levels of Folate and Homocysteine in DS cases with and without NTD*

Groups	**Neural Tube Defects**		
	Present (n=18)	Absent (n=90)	p value
Folic acid (ng/ml)	7.82 ± 2.36	8.15 ± 2.39	NS
Homocysteine (µmol/L)	4.76 ± 2.17	5.28 ± 2.44	NS

NS – Not significant

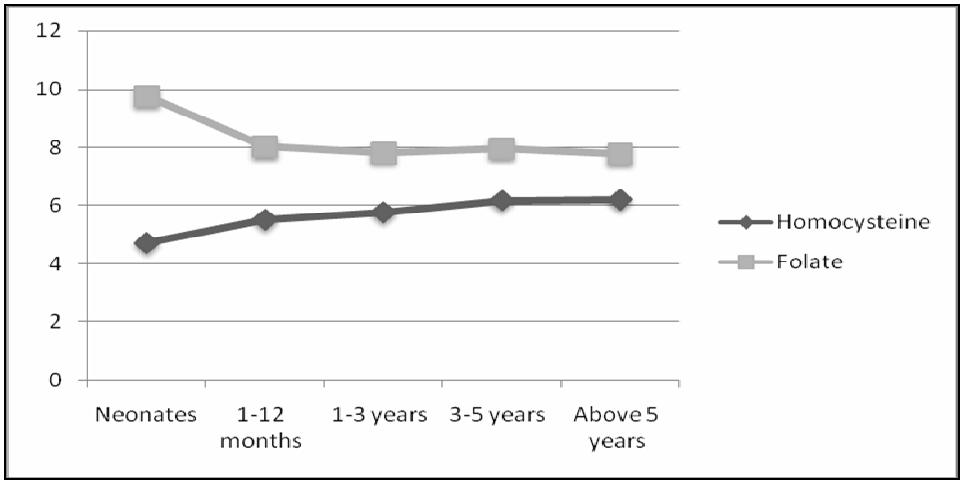

Figure 1. *Plasma levels of Folate and Homocysteine among DS cases in relation to age.*

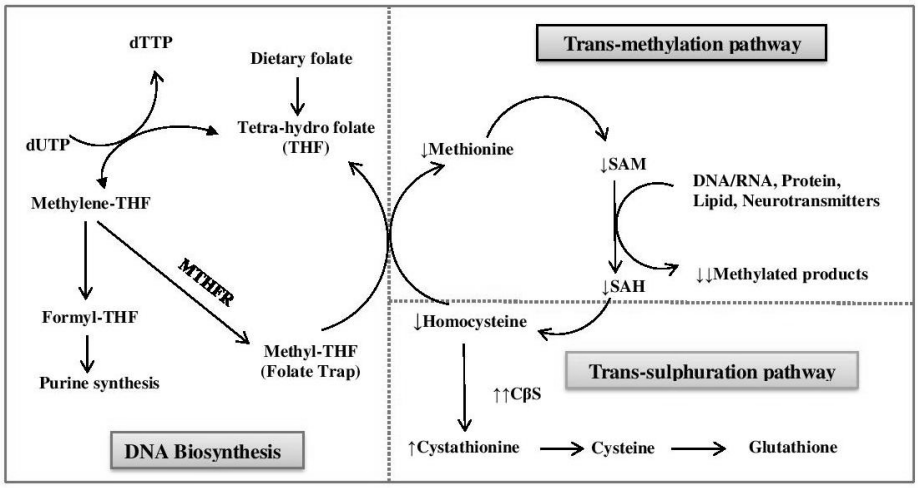

Figure 2. *Overview of Folate and Hcy pathway showing the effects of over-expression of CβS enzyme in DS individuals.*

Discussion

Disturbed/defective folate and homocysteine metabolism in DS has been put forward by several studies. It is clear from reports that over-expression of CβS in DS leads to functional folate deficiency. But, the data available on its effect on cardiovascular and nervous system in DS is scanty. Moreover, there are no studies which have attempted to probe the folate pathway in Indian children with Down syndrome. Hence, the objective of the present study is to estimate the plasma levels of folate and Hcy, study the influence of age on the said parameters and highlight the association between folate deficiency and CHD and NTD in DS.

Folic acid level in DS has been reported inconstantly with some authors suggesting normal [10,14] and some decreased levels [11,12]. Plasma folate in the current study was found to be in biological range and no statistical significance was observed between cases and controls. As expected, our results showed a significant decrease in the plasma Hcy levels in cases with DS which is in agreement with previous reports [5, 11-13]. A nearly two fold decrease in Hcy concentration in cases compared to controls presented in the current series is by far the lowest reported. From this point of view, hypohomocysteinemia observed here might be due to the increase in the transsulfuration pathway of Hcy, which results from the overexpression of cystathionine β-synthase on chromosome 21. It may promote a "folate trap" by decreasing the 5-Methyl-THF and remethylation of homocysteine to methionine and the subsequent cellular availability of tetrahydrofolate(THF) and S-adenosylmethionine (SAM) (Figure 2). Ultimately, 5-Methyl-THF is increasingly trapped because it can neither be converted to THF nor go back to Methylene-THF. Moreover, polymorphisms in Methylenetetrahydrofolate reductase (MTHFR) enzyme causing decrease in the conversion of 5-Methyl-THF further leads to non-availability of THF and SAM for biosynthesis reactions. It has therefore been suggested that

this gene-nutrient interactions affecting the one-carbon metabolism leads to "functional folate deficiency" contributing to the metabolic pathology in DS.

Influence of age on folate and homocysteine concentration in our study showed a decreasing and increasing trend respectively with advancement in age. This could be explained by high folate requirement in initial phases of growth and development in infants. Both folate and Hcy levels were decreased in cases with NTD. Although the decrease was statistically insignificant, the role of folate pathway in NTD cannot be ignored. No link between folate and CHD in DS could be established. But on the other hand, there was a very significant association in Hcy concentration among cases with and without CHD. Genetic polymorphism in MTHFR enzyme carrying 677 TT and 1298 AA mutant allele is associated with increase in homocysteine [15,16] levels and increased risk of CHD [17]. Hence, significantly increased levels of Hcy might have a genetic background which may play a role in the incidence of CHD in DS.

In conclusion, our study involving Indian children with DS, proves functional folate deficiency inspite of normal plasma levels of folic acid. Elevated Hcy concentration was observed with cases CHD but the exact mechanism for its association and the underlying genetic predisposition needs to be further studied. Supplementation with high doses of folic acid and B12 in patients with homocysteinemia has proved to be a successful in restoring normal homocysteine levels [18]). Since the study suggests that DS patients have low homocysteine levels and a high probability of a folate trap, we recommend intervention with folinic acid (5-formyltetrahydrofolate) which has several advantages. It is more efficiently absorbed as the reduced metabolite, rapidly polyglutamated and is more readily available for folate-dependent reactions than folic acid in DS subjects.

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