Serum cholinesterases in Down syndrome children before and after nutritional supplementation

Lakshmi K T, Surekha R H, Srikanth B, Jyothy A

ABSTRACT

Introduction: Down syndrome (DS) children have different degrees of developmental abnormalities associated with mental retardation. A cascade of pathological changes triggering alterations in cholinesterase-mediated functions seems to be the cause of neuronal and muscular dysfunctions, such as memory loss, disturbed cognitive skills, and language impairment in virtually all DS individuals, but there are currently no efficacious biomedical treatments for these central nervous system-associated impairments. The present study aimed to evaluate the effects of nutritional supplementation on cholinesterases in serum of DS children.

Methods: Activities of acetyl- and butyryl-cholinesterase were analysed in the serum samples of 40 DS children, along with an equal number of age- and sex-matched controls under study.

Results: The activities of serum acetyl- and butyrylcholinesterase were found to be low in DS children before nutritional supplementation, compared to controls, and showed considerable improvement after six months of supplementation of zinc in combination with antioxidant vitamins and minerals. A significant improvement was also observed in cognitive skills and behavioural patterns after nutritional supplementation.

Conclusion: The present pilot study suggests the significance of early intervention with nutritional supplementation in DS children to ameliorate the severity of this disorder.

Keywords: acetylcholinesterase, butyryl-cholinesterase, Down syndrome, nutritional supplementation, serum cholinesterase

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INTRODUCTION

Down syndrome (DS) is the most commonly-identified autosomal genetic disorder (trisomy 21), occurring in about one in 800 to one in 1,000 live births. Severe developmental delay and profound mental retardation are the hallmarks of phenotypic expression in DS patients. The brain of a child with DS develops differently from a normal one, attaining a form reduced in size and altered in configuration.[1] The trisomy 21 gene causes altered synaptic plasticity due to overproduction of S100, superoxide dismutase (SOD) and amyloid precursor protein (APP). The altered synaptic plasticity leads to synaptic loss, which leads to altered survival of the neuron and nerve growth factors. Abortive cell mitosis leads to amyloid apoptosis, which increases sensitivity of the neurons to produce neurotoxins.[2] Fiedler et al showed that higher gene dosage, inherent to the trisomic condition, affects cholinergic neurons in different regions of the central nervous system in a differential fashion.[3] Beccaria et al found that the cholinergic activity is precociously impaired in DS.[4]

Although DS individuals begin life with a normal complement of brain cholinergic neurons and cholinergic marker enzymes (acetyl- and butyrylcholinesterase), alterations in developmental pathways due to aberrant gene expression can impair cellular homeostasis and predispose to neurodegeneration of certain brain regions and types of nerve cells involving cholinergic transmission by shifting the balance toward a pro-apoptotic state.[5,6]

Many isomers of these cholinesterases are present in the body, and serum cholinesterases or pseudocholinesterases are one such class. They hydrolyse butyrylcholine and acetylcholine to thiocholine and choline, respectively. Becker stated that the phenotype of the brain in DS is different from that of a normal child, leading to mental retardation due to neuronal abnormalities, including alterations of cortical lamination, reduced dendritic ramifications, and diminished synaptic formation.[7] Earlier studies also showed that the dementia syndrome in DS is phenotypically similar to Alzheimer's disease (AD).[8]

Choline is not synthesised in the body and has to be derived from the diet. Therefore, to maintain the levels of this key substance, several therapeutic attempts are being made in individuals with AD. Hence, the present study

Institute of Genetics & Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad 500016, Andhra Pradesh, India

Lakshmi K T, MSc, PhD
Professor

Surekha RH, MSc, PhD
Scientist

Srikanth B, Msc, PhD
Research Scholar

Jyothy A, MSc, PhD
Director

Correspondence to:
Dr Jyothy Akka
Tel: (91) 40 2340 3681
Email: jyothycell@rediffmail.com

Table I. Activity of acetylcholinesterase in controls and DS children before and after nutritional supplementation.

Group	No. of cases	Mean ± SD (range) (U/ml)
Control	40	104.00 ± 20.05 (40–120)
Down syndrome		
Before supplementation	40	50.26 ± 12.00 (32–68)
After supplementation	36	96.00 ± 24.01 (32–110)

Control & DS before supplementation, $p < 0.0001$; DS before supplementation & after supplementation, $p < 0.0001$.

Table II. Activity of butyrylcholinesterase in controls and DS children before and after nutritional supplementation.

Group	No. of cases	Mean ± SD (range) (U/ml)
Control	40	6,258.00 ± 1,058.00 (3,200–7,200)
Down syndrome		
Before supplementation	40	3,840.76 ± 57.62 (2,722–4,815)
After supplementation	36	6,017.13 ± 1,353.24 (4,331–7,628)

Control & DS before supplementation, $p < 0.0001$; DS before supplementation & after supplementation, $p < 0.0001$.

aimed to evaluate whether serum cholinesterases reflect the cholinergic activity at the neuronal synapses of the DS children, and to study whether nutritional supplementation in these DS children has any effect on the activity of these enzymes. Our study also assessed cognition, memory, communication skills and learning abilities after six months of nutritional supplementation.

METHODS

This study was initiated upon receiving the approval from the ethical committee. Written consent was obtained from the parents and school authorities of normal and DS children. There were 40 children with DS, with ages ranging between five and 16 years. 29 boys, with a mean age of 10.08 years, and 11 girls with a mean age of 8.13 years, from the Special Education Centre for Mentally Handicapped, Institute of Genetics and Hospital for Genetic Diseases, Begumpet, Hyderabad, and Sweekar, Rehabilitation Centre and Special School for Handicapped Children, Secunderabad, Andhra Pradesh, were included in the study. 40 age- and sex-matched normal healthy children from the Government Centenary School, Secunderabad, Andhra Pradesh, were taken as controls for the present study. All the children were subjected to a careful clinical examination by qualified clinicians, and the data was recorded in special case proforma.

The nutritional supplementation dosage was formulated, in consultation with paediatricians of the Hospital and clinical nutritionists of Institute of Genetics, Hyderabad. It included zinc (as zincate of Yash Chemicals), calculated 1mg/kg body weight, in combination with antioxidant vitamins, multiminerals (as A to Z of Alchemy Chemicals), as per the recommended daily allowance (RDA) stipulation; the composition of each tablet was given as vitamin A (as acetate) Indian Pharmacopoeia (IP) 5,000 IU, vitamin E (as acetate) IP 25 IU, ascorbic acid IP 100 mg, thiamine mononitrate IP 10 mg, riboflavin IP 10 mg, pyridoxine hydrochloride 3 mg, cyanocobalmin IP 5 mcg, niacinamide IP 50 mg, folic acid IP 1 mg, calcium pantothenate IP 12.5 mg, cupricoxide equivalent to elemental copper 2.5 mg, sodium selenate equivalent to elemental selenium 60 mcg, manganese chloride equivalent to elemental manganese 1.4 mg, and chromium chloride equivalent to elemental chromium 5 mcg. Nutritional supplementation was administered for a period of six months in a single dose and the levels of serum acetyl- and butyrylcholinesterase were estimated before and after nutritional supplementation.

Activities of acetyl- and butyrylcholinesterase were analysed in the serum samples of subjects under study using the kits obtained from Sigma Chemicals Co. (St Louis, MO, USA). The assay was based on the principle that cholinesterase hydrolyses acetylcholine to acetic acid and choline. Formation of acetic acid caused a loss of m-Nitrophenol colour measured spectrophotometrically at 400–440 nm. The colour intensity was proportional to the cholinesterase activity in the sample. The assay was based on the principle that cholinesterase hydrolyses butrylcholine to butyric acid and thiocoline, that reacts with DNTB (5,5-dithiobis-2nitrobenzoic acid). This reaction liberates 5-thio-2-nitrobenzoic acid with the formation of a strong yellow colour measured at 405 nm. The rate of formation of the colour was directly proportional to the activity of cholinesterase in the sample. The analytical data was subjected to Student's *t*-test. p-values are shown below the Tables, with $p < 0.0001$ considered to be very significant, $p \leq 0.05$ being significant and $p > 0.05$ being not significant.

RESULTS

The values of butyryl- and acetylcholinesterase, in controls and in DS children before and after nutritional supplementation are shown in Tables I and II. The values of acetylcholinesterase recorded in DS children before

nutritional supplementation were found to be significantly low (50.26 ± 12.00 U/ml), when compared to controls (104.00 ± 20.05 U/ml). These levels were also found to be significantly increased after six months of nutritional supplementation (96.00 ± 24.01 U/ml) (Table I). The values of butyrylcholinesterase recorded in DS children before nutritional supplementation were found to be low (3,840.76 ± 57.62 U/ml), when compared to controls (6,258.00 ± 1,058.00 U/ml). These levels were found to be significantly increased after six months of nutritional supplementation (6,017.13 ± 1,353.24 U/ml) (Table II).

DISCUSSION

The morphology of the brain in a DS child is different from that of a normal child, both in its size and altered gyral configuration. DS children have different degrees of developmental abnormalities associated with mental retardation, as the extra chromosome 21 regulates maturation delay of synaptogenesis and cortical dysgenesis. Ultrastructurally in DS, the synaptic density, synaptic length and contact zones were found to be abnormal. According to Becker, synaptogenesis can be affected by several factors, such as errors in neuronal proliferation, migration and differentiation, and these minor alterations could account for the structural basis of the clinical manifestations.[7] The altered synaptic plasticity is due to overproduction of S100 APP and SOD, which leads to synaptic loss through an array of reactions causing increased sensitivity of the neurons to neurotoxins that culminate in apoptosis.[2,6]

A cascade of pathological changes triggering alterations in cholinesterase-mediated functions seem to be the cause of neuronal and muscular dysfunctions, like memory loss and disturbed cognitive skills. Therapies used for AD to improve cognitive function in people with mild to moderate disease, affect levels of acetylcholine in the body. The body makes acetylcholine out of the nutrient choline. It is known that cholinesterases catalyse the hydrolysis of choline esters—acetyl and butyryl—to choline and thiocholine, where choline is responsible for the myelinisation of neurons. Acetylcholinesterase activity is found to be lower in most regions of the AD brain, but increased within and around amyloid beta plaques.[9] Changes in acetycholinesterase isoforms and glycosylation patterns in AD brain may be the direct consequences of altered APP metabolism. According to Isacson et al, DS-related memory loss could also be triggered by alterations in APP processing or acetylcholinesterase-mediated neuronal function, or both, which in turn triggers the over-expression of amyloid beta, synaptic malfunction that eventually leads to dendrite loss with age.[10]

The hydrolytic activity of cholinesterases on esters like acetylcholine and butyrylcholine, and other isomers is reduced in DS children as compared to normal children.[11] Neuromotor dysfunction, cognitive and language impairment are observed in virtually all DS individuals, but currently, there is no efficacious biomedical treatment for these central nervous system-associated impairments.[12] According to Temple et al, the environmental intervention aimed at improving the level of cognitive functioning seems to be useful in deferring the onset of dementia in DS individuals.[13] Kish et al suggested that DS individuals begin life with a normal complement of brain cholinergic neurons.[5] This opens the possibility of early therapeutic intervention to prevent the development of brain cholinergic changes in them. The role of zinc in the brain has received attention in the past two decades, as it is important to the function of a number of enzymes and proteins unique to the brain and it is vital to neurotransmission.

In the present study, the activities of the cholinesterases in the serum (both serum acetyl- and butyrylcholinesterase) which were low in DS children before supplementation, compared to controls, showed considerable improvement after six months of supplementation with zinc, in combination with antioxidant vitamins and minerals. It can be inferred that the cholinergic activity of the neurons in the brain might have been improved to result in a reduced neuropathology in the DS children, as evidenced by psychological assessment of these children, which revealed significant improvement in cognitive skills and behavioural patterns after nutritional supplementation. Similarly, Turkel claimed that a mixture of 48 different ingredients could improve the intelligence and the appearance of DS children.[14]

Earlier studies have administered nutritional supplementation for varying periods and recorded improved cell-mediated immunity, leucocyte chemotaxis, improved response of lymphocytes to mitogens, a trend towards fewer episodes of cough and fever, decreased incidence of infectious diseases due to increased efficiency of the immune system, and normalisation of endocrine parameters, etc.[15-22] The positive outcome of the present study, as evidenced by the clinical and psychological assessments, showed that the neuronal stress in DS children was addressed encouragingly by the nutritional supplementation. Hence, the present study suggests the significance and importance of early intervention with nutritional supplementation in DS children to ameliorate the severity of this condition.

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