

Antioxidant enzyme activity in children with Down syndrome.

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Abstract

Down syndrome or trisomy 21 is the single most common genetic cause of mental retardation. It has been postulated that there is oxidative stress in Down syndrome due to over expression of superoxide dismutase 1 (SOD -1), an antioxidant enzyme which is coded on chromosome 21(21q22.1). The present study included 31 clinically diagnosed children with Down syndrome. The diagnosis of trisomy 21 was confirmed by chromosomal analysis and activity of antioxidant enzymes like erythrocytic SOD (SOD-1), erythrocytic catalase (CAT) and erythrocytic glutathione peroxidase (GPx) were estimated. The results of biochemical analysis showed elevated activities of SOD-1, GPx and decreased activity of catalase. The variations in the activities of antioxidant enzymes were statistically not significant, whereas the ratio of SOD -1 to CAT and GPx (SOD-1/CAT+GPx) was significantly elevated indicating oxidative stress in Down syndrome.

Key Words: Down syndrome, Oxidative stress, Chromosomal analysis, Superoxide dismutase 1 (SOD -1), Catalase (CAT), Glutathione peroxidase (GPx)

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Introduction

Down syndrome (Trisomy 21) is the most common autosomal aneuploidy disorder in children affecting one in 800 live births [1]. There are evidences supporting oxidative stress in Down syndrome [2-4]. It has been proposed that the oxidative stress in Down syndrome is due to over expression of superoxide dismutase 1 (SOD -1), an antioxidant enzyme which is coded on chromosome 21(21q22.1). Several studies have shown elevation in the activity of Cu -Zn superoxide dismutase (SOD-1) by 50% in cases with Down syndrome [5-7]. The over expression of SOD -1 is due to the phenomenon of gene dosage effect as a result of which the activity of other antioxidant enzymes like catalase (CAT) (8-12) and glutathione peroxidase (GPx) (2,11-13) harbouring in chromosome 11p13 and 3p21.3 respectively are also altered. It is considered that the imbalance in the activities of antioxidant enzymes will lead to the accumulation of hydrogen peroxide which causes oxidative damage. Hence the elevated ratio of SOD 1 to CAT and GPx favouring oxidative damage in Down syndrome compared with controls is important rather than the elevation of individual antioxidant enzymes [2,8]. The present study aims to evaluate the activity of enzymatic antioxidants in children with Down syndrome.

Material and Methods

The study group consisted of 31 clinically diagnosed Down syndrome children between 3 months and 14 years (mean \pm SD, 3.64 ± 3.39 yrs) of age with 18 males and 13 females. The control group consisted of same number of age and gender matched normal children. Children with infections and other severe illness were excluded. Based on the age both cases and controls were divided into three groups, Group I: less than 4 yrs (n=19), Group II: 4 to 8 yrs(n=06) and Group III : more than 8 years (n=06). The study was approved by Institute Ethics Committee. Informed written consent was obtained from the parents of the study and control groups. Anticoagulated blood samples were collected by venipuncture. The clinical diagnosis of Down syndrome was confirmed by conventional lymphocyte cell culture [14] and the chosen metaphase spreads were screened using automated karyotype software, *Ikaros Metasystem of Carl Zeiss, Germany*. Separated RBC pellets were used for the estimation of erythrocytic superoxide dismutase 1, catalase and glutathione peroxidase. SOD -1 activity was determined by standard *RANSOD kit Cat. No. SD 124 from RANDOX U.K.* using *AU 400 auto analyser*,

Olympus, Japan. Erythrocytic catalase activity was assayed by the method of Aebi H 1984 [15], which is based on monitoring the rate of decomposition of hydrogen peroxide spectrophotometrically at 240 nm and the activity of erythrocytic glutathione peroxidase was estimated by the method of Wendel et al 1981 [16], by monitoring the decrease in concentration of reduced glutathione (GSH) in the presence of hydrogen peroxide spectrophotometrically at 412 nm.

The parametric *Unpaired t test* and non parametric *Mann Whitney test* were used to estimate differences between cases and control group at a significance threshold of $p < 0.05$. All statistical tests were performed using *Graph-Pad inStat 3 software*.

Results

Chromosomal analysis showed pure trisomy 21 in all 31 cases in the study group. Other variants of Down syndrome like translocations, mosaicism and partial trisomy were not observed.

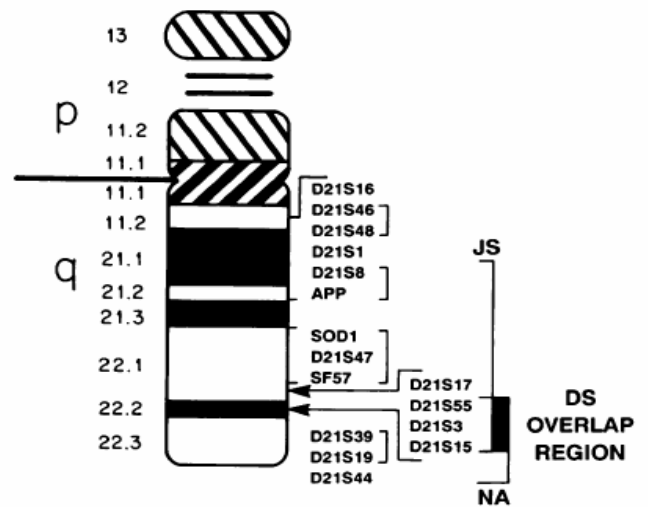


Fig 1: Chromosome 21 showing SOD 1 gene locus 21q22.1 (17)

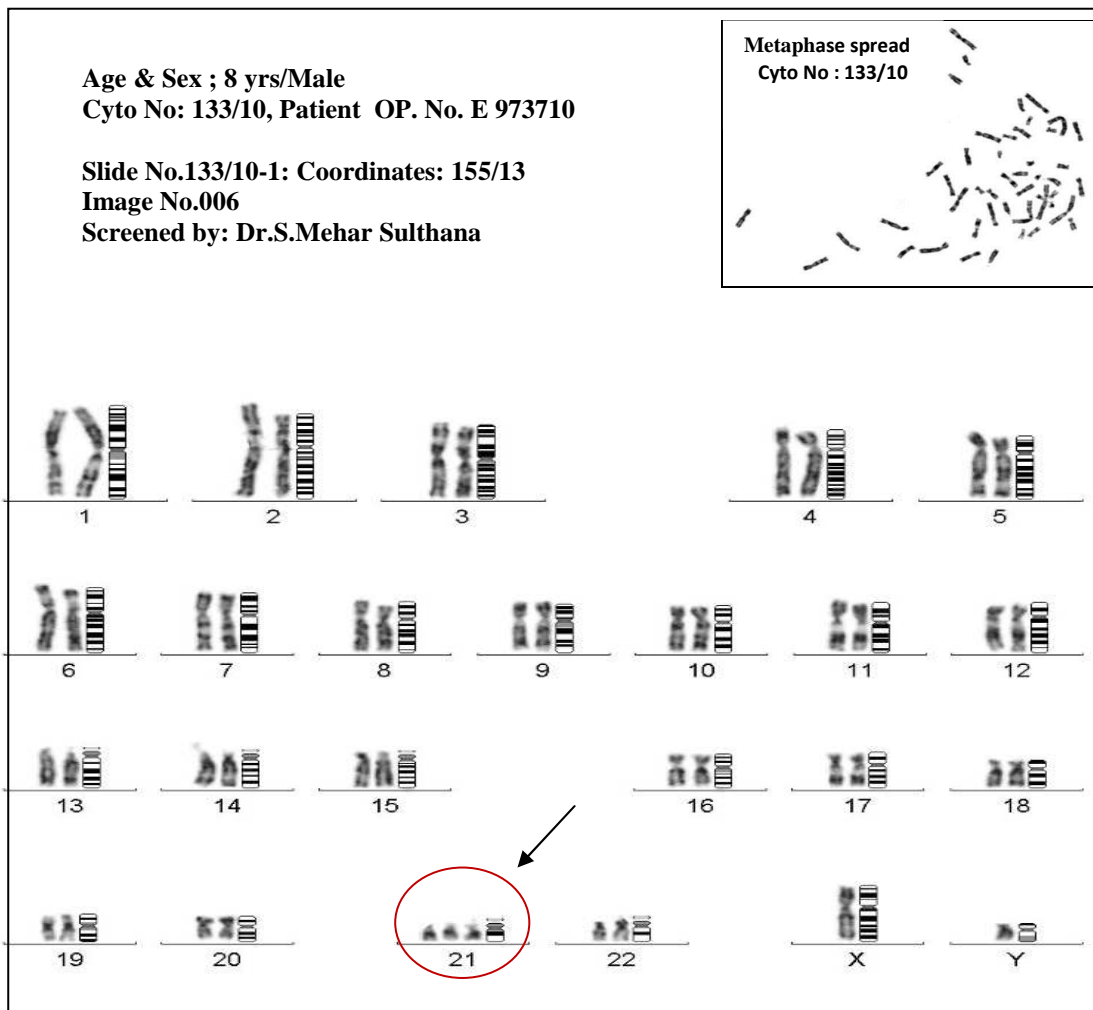


Fig 2: Karyotype of trisomy 21 matched with ideogram

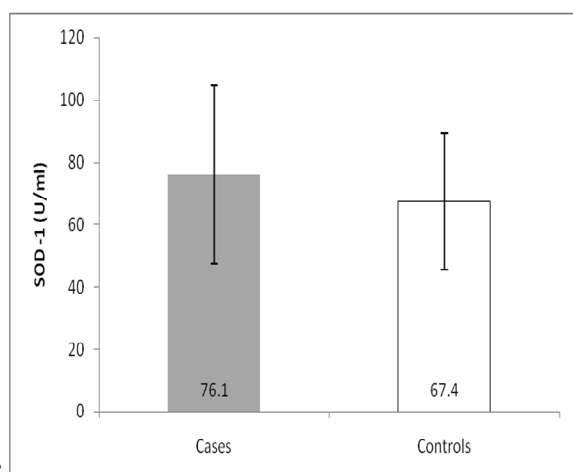


Fig. 3

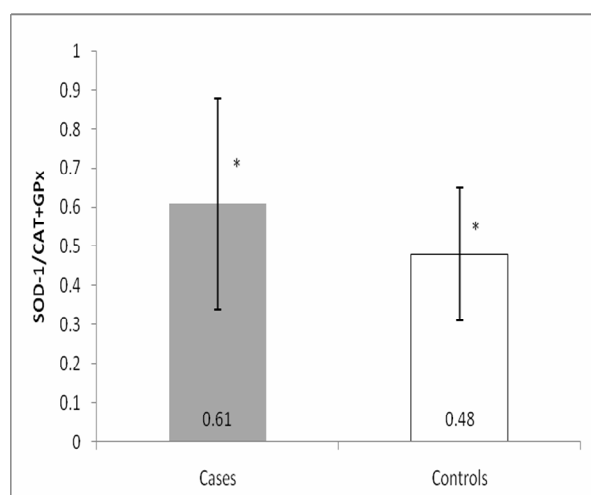


Fig 6: Showing the ratio of SOD1 to catalase and GPx in cases compared with controls (* p value <0.05)

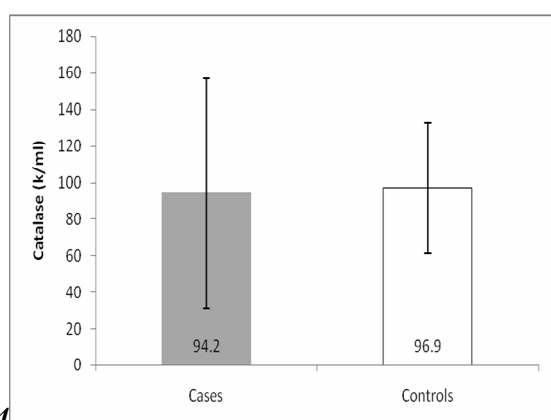


Fig. 4

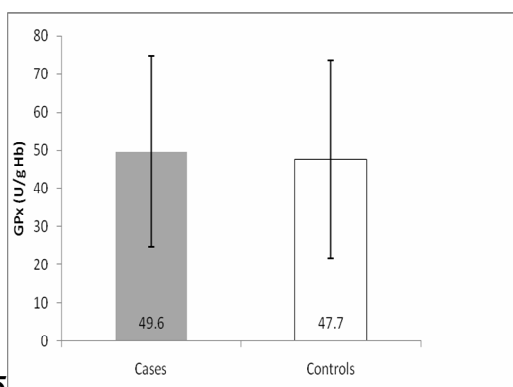


Fig. 5

Figures 3,4,5: Showing the activity of SOD-1, catalase, GPx respectively in cases compared with controls

The results of biochemical analysis showed elevated activities of SOD-1, GPx and decrease in the activity of catalase. The variations in the activities of antioxidant enzymes were statistically not significant. Whereas the ratio of SOD -1 to CAT and GPx (SOD-1/CAT+GPx) was significantly elevated in children with Down syndrome when compared to controls (Table 1).

At the level of individual age groups, group I and II showed increase and decrease of SOD 1 and catalase activities respectively in cases with Down syndrome when compared with controls. Whereas group III showed decrease and increase of SOD 1 and catalase activities respectively in cases when compared with controls. GPx activity in cases were almost equal to controls in Group I and III, whereas Group II showed elevation. The variations in the activities of antioxidant enzymes were statistically not significant .The ratio of SOD-1 to catalase and GPx was significantly elevated in children with Down syndrome below four years(Group I).The activity of SOD - 1 and catalase decreases with age till 8 yrs and thereafter it increases in both case and control groups (Table 2).

Table 1: Antioxidant enzyme activity in cases with Down syndrome and controls

Oxidative stress parameters	Cases (n =31)	Controls(n = 31)
SOD 1(U/ml)	76.1 ± 28.5	67.4 ± 21.8
GPx (U/g Hb)	49.6 ± 25.1	47.4 ± 26
CAT (k/ml)	94.2 ± 63	96.9 ± 35.6
SOD 1/CAT+ GPx	0.61 0.27*	0.48 ± 0. 17*

*p value <0.05

Table 2: Antioxidant enzyme activity in different age groups among cases and controls

Enzymatic antioxidants	Age Groups	Cases	Controls
SOD – 1 (U/ml)	Group I	75.4 ± 32.4	60.4 ± 14.4
	Group II	68.5 ± 17.4	65.8 ± 18.3
	Group III	86.1 ± 24.3	91.8 ± 27.7
GPx (U/g Hb)	Group I	42.6 ± 20.5	42.21 ± 24.6
	Group II	78.2 ± 30.6	66.7 ± 25.3
	Group III	43.3 ± 10.5	44.1 ± 26
CAT (k/ml)	Group I	98.5 ± 75.4	101.1 ± 38.5
	Group II	76.5 ± 46.1	86.3 ± 33.9
	Group III	98.7 ± 37.6	92.3 ± 29.6
SOD -1/CAT+GPx	Group I	0.64 ± 0.29*	0.44 ± 0.16*
	Group II	0.49 ± 0.20	0.43 ± 0.11
	Group III	0.64 ± 0.27	0.66 ± 0.18

* *p* value < 0.05

Discussion

Several studies in Down syndrome have shown elevation in the activity of SOD-1 by fifty percent [5-7], decrease in few [13] and normal SOD 1 activity in some [18,19]. In the current series, activity of SOD 1 was elevated (statistically not significant) in cases with Down syndrome which were confined to the group I and II. The sustained increase of SOD-1 activity in cases upto 19 yrs of age when compared to controls [11], was not seen in the present. The increase of SOD-1 activity compared to controls was confined upto 08 years of age group. Thereafter a decrease was observed in the group III which were of 8-14 yrs of age. Cases with older age group (20-50 yrs) of Down syndrome in earlier studies showed a remarkable decrease of SOD-1 activity [11] was not seen in the present series as the study group is confined only upto 14 yrs.

In the present series, the SOD-1 activity among the cases surprisingly showed an elevation in group I with decline in group II followed by an elevation again in group III. The present trend of increase followed by decrease and subsequently an increase with age was not reported earlier. The trend needs further investigation with larger number of cases of similar age groups.

The phenomenon of oxidative stress in Down syndrome has been explored by yet another approach through catalase activity in children and adults with varied opinions [8-12]. Elevated [10-12] and low catalase activity [9] in Down syndrome have been reported. In the current series, the catalase activity was found to decrease (statistically not significant) in cases when compared to controls. There seems to be an imbalance in the activity of SOD-1 and catalase in the present series. The imbalance being increa-

ased activity of SOD-1 and decreased activity of catalase. The imbalance indicates the existence of oxidative stress in cases investigated.

There are further evidences for oxidative stress and undesirable reactions as a result of accumulation of reactive oxygen species in individuals with Down syndrome. Elevated GPx activity have been identified by many individuals with trisomy 21 of various age groups and multi-system involvement [2,8,11,12]. The present series of trisomy 21 which was exclusively of paediatric age group having physical disability and systemic involvement such as hypothyroidism and heart defects and also of low cognitive performance showed statistically insignificant elevation of Gpx activity.

The elevated SOD -1 levels in the present series could be the reason for the elevated levels of GPx. While looking at the profile of a complete antioxidant enzyme activities in the present series (SOD-1, Catalase, GPx), there appears to be insignificant difference in enzyme activity of each indicating elevated oxidative stress in Down syndrome.

It is convention to correlate the said enzymes by means of ratio between Superoxide dismutase-1 (SOD-1) and Catalase + Glutathione peroxidase (GPx) collectively since 1998. The elevated ratio of SOD-1 to Catalase + GPx was observed in Down syndrome when compared to controls [2,8]. Also, there are reports of normal values with reference to the said ratio in cases of Down syndrome [11]. In thirty one cases investigated in the present study, a statistically significant elevated ratio was observed between SOD-1 versus catalase and GPx activity. Increase of SOD -1 activity by fifty percent in cases compared to controls in children and adults has been noted in earlier

studies [5-7]. On application of 'antioxidant enzyme activity ratio, a 12.5 fold increase was noticed in the present investigation among children below 14 years of age. Children with Down syndrome have elevated levels of oxidative stress. Hence, a therapeutic trial of antioxidants in patients with Down syndrome may be beneficial in reducing morbidity.

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