

ANTIOXIDANT ENZYMES STATUS AND THYROID STIMULATING HORMONE LEVEL OF DOWN SYNDROME PATIENT IN WEST BENGAL, INDIA: A CASE-CONTROL STUDYGARGI PODDER*¹, ANIKET ADHIKARI¹, AULEY DE¹, JAYSHREE BANERJEE², MADHUSNATA DE²¹Research Scholar, ²Professor Department of Genetics, Ramakrishna Mission Seva Pratishthan, Vivekananda Institute of Medical Sciences, 99 Sarat Bose Road, Kolkata-700026. Email: gargi.podder86@gmail.com

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ABSTRACT

Objective: The copper-zinc superoxide dismutase gene resides on chromosome 21 and is over expressed in Down syndrome patients. Reactive oxygen species can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired. Down syndrome is associated with various forms of thyroid dysfunction, hypothyroidism being the most common. The additive effects of both co-morbid conditions lead to further amplification of the clinical problems in the children with Down syndrome. So the objective of the current study is to evaluate the activity of some antioxidant enzymes like superoxide dismutase and glutathione peroxidase and level of thyroid stimulating hormone among Down syndrome cases and then compare with healthy control group.

Methods: The present work aimed to study the changes in the quantitative *in vitro* activities of the antioxidant enzymes – glutathione peroxidase and superoxide dismutase in the erythrocyte and thyroid stimulating hormone level of the study group. This study group included both Down syndrome patients and age, sex matched healthy controls.

Results: It was found that significant increase in the activities of both glutathione peroxidase and superoxide dismutase and thyroid stimulating hormone level in patients as compared with healthy controls. This study has revealed that increased antioxidant enzymes activity and thyroid stimulating hormone level are the three significant indicators of Down syndrome patients.

Conclusion: Study of biochemical parameter reveals that Thyroid stimulating hormone level, Glutathione peroxidase and Superoxide dismutase activity is increased among DS cases.

Keywords: Down syndrome, Glutathione peroxidase, Superoxide dismutase, Thyroid stimulating hormone, Trisomy 21.

INTRODUCTION

Down syndrome or Down's syndrome (DS) is the most common chromosomal disease, consisting of a trisomy of the 21st chromosome, occurring in 1 in 600 to 1 in 800 live births [1]. The distal segment of this chromosome is the site of the gene encoding the synthesis of SOD. It has been suggested that the excess of genetic information in DS patients is the cause of an increase in the activity of SOD1 in these patients [2,3]. It has been hypothesized that an increase in oxidative stress in patients with DS would account for the appearance of different diseases, such as atherosclerosis, accelerated cell aging, cellular mutagenicity, and the neurologic disorders that often occur in these patients [4].

Reactive oxygen (RO) species are substances that are released during oxidative metabolism. RO species include the superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and the hydroxyl radical (OH[•]). The reactions of RO species with macromolecules can lead to DNA mutations, changes in the structure and function of proteins, and peroxidative damage of cell-membrane lipids [5]. There is a primary defense system against oxidative stress, mediated by sequential enzymatic reactions. In the first step of the process, CuZn-superoxide dismutase (SOD1) catalyzes the dismutation of O₂ to H₂O₂. Glutathione peroxidase (GPx) then independently converts H₂O₂ to water [6]. Any increase in SOD1 catalytic activity, therefore, produces an excess of H₂O₂ that must be efficiently neutralized by either GPx or catalase [7].

Several groups have proposed that many of the neurologic symptoms of Down syndrome result from the increased intracellular activity of cytosolic copper/zinc superoxide dismutase (Sod1), an enzyme coded on chromosome 21 (21q22.1), which converts the superoxide radical into hydrogen peroxide. Sod1 is a constitutive enzyme with activity that is increased by 50% in patients with trisomy 21. Supporters of the hypothesis of Sod1-induced damage in Down syndrome assumed that increased Sod1 activity causes an imbalance in the steady-state oxidative stress that increases formation of hydrogen peroxide and other oxidants such as the hydroxyl radical [4]. Erythrocytes are perform in an environment in

which they are constantly exposed to both extracellular and intracellular sources of reactive oxygen species. The conversion of oxyhemoglobin to methemoglobin inside the erythrocyte results in the concomitant production of O₂⁻. Oxidative damage can alter membrane components and, consequently, erythrocyte membrane fluidity [8].

The most common autoimmune disease in Down syndrome is related to the thyroid gland. Thyroid auto-antibodies are found in 13–34% of patients with Down syndrome [9]. The clinical symptoms and signs of both Down syndrome and hypothyroidism are overlapping to some extent e.g. hypotonia, lethargy, dullness, mental retardation, growth failure, prolonged neonatal jaundice, delayed closure fontanelles, macroglossia, obesity etc. Thus, both hypothyroidism and hyperthyroidism are more common in patients with Down syndrome than in the general population [10].

This work aims to evaluate the levels of superoxide dismutase and glutathione peroxidase activities and serum level thyroid stimulating hormone (TSH). A possible erythrocytic lysis and the consequent leaking of enzymes, erythrocyte osmotic fragility, and haemograms of people with Down syndrome were evaluated.

PARTICIPANTS AND METHODS**Study group**

Our study group comprised of 115 individuals, which include both Down syndrome patients (n=85) and age, sex-matched healthy control (n=30). The studied population belonged to ages ranging from 2 days to 30 years. Among the patients studied, 55 were male and 30 were female individuals. Among the control population studied, 17 were male and 13 were female individuals. The studied population came from mainly lower socio-economic strata of the society. So, maximum patient's parental literacy and family income were very low. Cause of that, they are not properly aware about genetic abnormality. Down syndrome individuals were distributed according to their age.

Ethical Clearance

The studies involving human subjects were reviewed and approved by the ethical Committee of the Vivekananda Institute of Medical Sciences. All individuals were included in this study only after the Informed Consent of their parents.

Estimation of glutathione peroxidase activity

This method is based on Paglia and Valentine (1967) [11]. Glutathione Peroxidase catalyzes the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340nm is measured. Quantitative *in vitro* determination of glutathione peroxidase activities in whole blood were estimated with 0.05ml heparinized whole blood using Ransel glutathione peroxidase assay kit (Randox, United Kingdom). The samples were assayed by UV-Visible spectrophotometer (Spectronic, USA) at a wavelength of 340nm. Appropriate negative and positive controls were maintained with each batch of estimation.

Estimation of Superoxide Dismutase Activity

This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye [12]. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T. under the conditions of the assay. Quantitative *in vitro* determination of superoxide dismutase activities in whole blood were estimated with 0.5ml heparinized whole blood using Ransod superoxide dismutase assay kit (Randox, United Kingdom). The whole blood was centrifuged at 3000 rpm for 10 mins followed by aspiration of plasma to obtain the erythrocytes which were washed with 0.9% NaCl solution. The erythrocytes were made upto 2ml with cold redistilled water followed by incubation at 4°C. The lysate was assayed by UV-Visible spectrophotometer (Spectronic, USA) at a

wavelength of 505 nm. Appropriate negative and positive controls were maintained with each batch of estimation.

Estimation of Serum level TSH

Total serum was separated out and kept in a clean sterile micro centrifuge tube (1.5ml Tarson). Thyroid Stimulating Hormone (TSH) was estimated by Vitros Eci,Ortho Clinical Diagnosis (Jonson & Jonson), autoanalyser from the blood serum.

Erythrocyte osmotic fragility Test

It was assessed by adding 0.05 mL of fresh whole blood to various concentrations of aqueous sodium chloride and incubating the mixture for 15 minutes at room temperature. After incubation, the mixtures were centrifuged, and the percentage of hemolysis was determined spectrophotometrically at 540 nm by measuring the absorbance of released hemoglobin [13]. The fragility was considered to have increased when at least 1 of 12 dilutions resulted in an elevation of the hemolytic values to the sodium chloride concentration used.

Complete Haemogram

The Haemoglobin (Hb), Total count of WBC, Total count of RBC, Platelet count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin concentration (MCHC) were estimated by Sysmex K-2000, automated cell counter.

Statistical analysis

Statistical analyses of the experiments were done by Student's *t* test.

RESULTS AND DISCUSSION

Quantitative *in vitro* glutathione peroxidase (GPx) activity and superoxide dismutase (SOD) activity and TSH level were estimated in both patients and healthy controls (Table 1).

Table1: Glutathione Peroxidase and superoxide dismutase activity of the Down syndrome patients and control group

Types	No. of individuals	Glutathione peroxidase activity (U/g Hb) Mean±S.E	Superoxide dismutase activity (U/g Hb) Mean±S.E	Thyroid stimulating hormone (mIU/ml) Mean±S.E
Healthy control	30	23.56±3.28	1221.46±13.23	3.79±2.47
Patient	85	47.14±1.06*	1875.79±40.46*	10.67±0.897*

*Statistically significant at $P \leq 0.001$ (student *t* test)

In current study glutathione peroxidase activity showed about 2 fold increase as compared to healthy individuals ($p \leq 0.001$). Glutathione peroxidase activities and Superoxide dismutase were studied in different age group of patients (Table 2).

Table2: Glutathione Peroxidase, Superoxide dismutase activities and Thyroid stimulating hormone levels according to different age group of the Down syndrome cases

Age	Groups	Total No. of individuals	Male % present	Female % present	Glutathione peroxidase activity (U/g Hb) Mean±S.E	Superoxide dismutase activity (U/g Hb) Mean±S.E	Thyroid stimulating hormone (mIU/ml) Mean±S.E
< 12 months	I	44	37.65	14.12	41.92±1.47	1851.56±65.26	13.33±1.43
1 yr-4 yr 11 months	II	24	15.29	12.94	46.38±1.84	1789.74±61.004	6.24±1.39
5 yr- 9yr 11 months	III	6	2.35	4.71	43.95±3.49	1978.85±98.74	7.38±2.59
10yr-14yr11 months	IV	2	2.35	0	48.63±2.92	1762.31±81.15	9.56±3.38
15yr-19yr11 months	V	5	4.71	1.17	49.214±1.81	1978.31±167.82	10.13±4.55
> 20 yrs	VI	4	2.35	2.35	54.735±4.45	1860.59±90.32	14.75±1.33

It was observed that >20 years age group of patients showed the highest glutathione peroxidase activity values (54.735±4.45 U/g Hb) among all age groups. In this study superoxide dismutase activity of the patients showed about 1.5 fold increase as compared to healthy

individuals ($p \leq 0.001$). Group V (15 yr- 19yr 11 month's age) patients showed the highest superoxide dismutase activity values (1978.31±167.82 U/g Hb) among all groups of patients.

The serum level Thyroid Stimulating Hormone showed about 3 fold increase as compared to healthy individuals ($p \leq 0.001$).

Serum level Thyroid Stimulating Hormone was also studied in different age group of patients (Table 2). It was observed that >20 years age group of patients showed the highest serum level TSH values (14.75 ± 1.33 mIU/ml) among all age groups.

The detailed hematological profile and fragility of red blood cell were studied by hemogram (Table 3) and erythrocyte osmotic fragility (Table 4) test. Although individuals with Down syndrome have had a slightly increase erythrocyte osmotic fragility than control group, the difference was statistically insignificant. These results, along with the normal hemogram values, indicate that there was probably no erythrocyte lysis in the Down syndrome group.

Table 3: Complete haemogram of the study group

Types	No. of individuals	Total count of WBC ($\times 10^3/\mu\text{l}$) Mean \pm S.E	Total count of RBC ($\times 10^6/\mu\text{l}$) Mean \pm S.E	Hb (g/dl) Mean \pm S.E	HCT (%) Mean \pm S.E	MCV (fl) Mean \pm S.E	MCH (pg) Mean \pm S.E	MCHC (g/dl) Mean \pm S.E	Platelet count ($\times 10^3/\mu\text{l}$) Mean \pm S.E
Healthy control	30	10.04 \pm 0.8	4.92 \pm 0.3	12.54 \pm 0.15	38.02 \pm 0.78	86.44 \pm 1.41	23.7 \pm 0.45	30.34 \pm 0.9	280.5 \pm 14.3
Patient	85	8.83 \pm 0.25	4.49 \pm 0.2	10.61 \pm 1.03	39.38 \pm 0.65	71.48 \pm 0.86	28.09 \pm 0.54	34.2 \pm 0.5	241.65 \pm 4.96

Table 4: Erythrocyte Osmotic Fragility test of the studied population

Types	No. of individuals	0.2% NaCl Concentration Mean \pm S.E	0.3% NaCl Concentration Mean \pm S.E	0.4% NaCl Concentration Mean \pm S.E	0.5% NaCl Concentration Mean \pm S.E	0.6% NaCl Concentration Mean \pm S.E
Healthy control	30	0.322 \pm 0.054	0.452 \pm 0.22	0.691 \pm 0.075	0.848 \pm 0.09	0.943 \pm 0.12
Patient	85	0.639 \pm 0.032	0.708 \pm 0.033	0.944 \pm 0.034	1.11 \pm 0.035	1.286 \pm 0.04

Many papers have reported investigations of oxygen metabolism in trisomy 21 patients (Down syndrome) and there have been suggestions that some of the pathological symptoms of this disease, for example premature ageing, or specific brain lesion, result from the increase of the oxidative damage inside the cells [14,15]. One of the observed and probable defense mechanisms of the cell against the oxidative damage is the increase of catalytic activity of enzymes superoxide dismutase, and glutathione peroxidase counteracting toxic oxygen derivatives [15,16]. Glutathione peroxidase catalyses the reduction of hydrogen peroxide and organic hydroperoxides to alcohols of lower toxicity, thus forming the first line of defense against peroxidative damage of unsaturated lipids [17]. The gene for glutathione peroxidase synthesis is situated on chromosome no. 3 and for this reason the increase of the glutathione peroxidase activity in trisomy 21 patients is not caused by a gene dosage effect [18]. The higher activity of the enzyme in the cells may be explained as one of the mechanisms controlling the response to an acceleration of oxidative processes inside the trisomic 21 cells.

The results of biochemical analysis showed elevated activities of GPx and SOD. The variations in the activities of antioxidant enzymes were statistically significant ($p \leq 0.001$).

The increase of glutathione peroxidase activity in erythrocytes is the secondary result of a regulatory mechanism involving an increased activity of superoxide dismutase and intensified production of H_2O_2 in trisomy 21 cells [19]. Elevated glutathione peroxidase activity in Down syndrome patients could be a useful protective mechanism against peroxidative cell damage.

Several studies have shown elevation in the activity of Cu -Zn superoxide dismutase (SOD-1) by 50% in cases with Down syndrome [20,21,22]. 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) [23] and glutathione peroxidase (GPx) [24] 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 GPx favoring oxidative damage in Down syndrome compared with controls is important rather than the elevation of individual antioxidant enzymes [24,25]. Frants et al. observed DS patients showed an increase of about 50% in SOD-1 activity due to higher levels of SOD-1 protein 21 which is the result of extra copy of genetic material in the cell. Other Down syndrome

disorders are based on the duplication of the same subset of genes (e.g. various translocations of chromosome 21) [26].

Similar results were reported by Goyal et al. (2010) [27]. The SOD levels in blood were significantly higher ($p=0.008$) in children with Down syndrome (mean=313.7IU/ml) as compared to the control group (mean=140.4IU/ml). The findings indicate dyslipidemia and increased SOD activity in individuals with Down syndrome. The raised antioxidant activity of SOD, because of over expression of genes situated on chromosome 21, probably offers some protection against the development of atherosclerosis despite the occurrence of dyslipidemia. The current study may contribute towards a better understanding of the importance of antioxidants in protection against atherosclerosis in hypertriglyceridemias. Patients with Down syndrome have elevated levels of oxidative stress. Antioxidants have been shown to prevent the destruction of β -cells by inhibiting the peroxidation chain reaction [28]. Hence, a therapeutic trial of antioxidants in patients with Down syndrome may be beneficial in reducing morbidity. Transient hypothyroidism is the most common form of thyroid dysfunction observed in Down syndrome patients. Mark Selikowitz (1993) [29] had observed in his longitudinal study that 40% of these cases of compensated hypothyroidism resolved spontaneously. Gibson et al (2005) [30] observed that 47% of subclinical hypothyroid Down syndrome patients were subsequently found to have normal TSH levels after a gap of four to six years.

The acquired form of hypothyroidism is usually associated with thyroid antibodies of different types and is more common in children above 8 years. Hypothyroidism was observed in 20% of the cases between 9 - 12 years [30].

Hypothyroidism develops in one third of patients with Down syndrome before the age of 25 years. Autoimmune thyroid disease is uncommon in preschool children with Down syndrome, but occurs commonly after the age of 8 years. Because symptoms of hypothyroidism might be mistaken for symptoms related to the natural course of Down syndrome, so it is important to screen annually for thyroid function [31].

In our study, the Down syndrome group had statistically significant ($p \leq 0.001$) increase in TSH levels compare to control. A comparison was made among the different age groups of patients with respect to the Thyroid Stimulating Hormone (TSH) level. It was found that the level of TSH was highest in the age group above 20 years among all age group of patients.

TSH levels were found to have a positive correlation with blood pressure [32]. Reasons for the condition's heterogeneity were clarified by the discovery of thyroid stimulating hormone receptor blocking and enhancing immunoglobulins, which affect selectively hormone synthesis and thyroid gland growth [33].

CONCLUSION

The findings of this study indicated there is increased SOD and GPx activity in patients with Down syndrome. The raised antioxidant activity of SOD, because of over expression of genes situated on chromosome 21, probably offers some protection against the development of atherosclerosis and raised GPx activity of neutralizing increased activity of SOD enzymes. The current study may contribute towards a better understanding of the importance of antioxidants in Down syndrome and also the need to re-assess treatment strategies in case of those patients.

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