

Oxidative stress in Down Syndrome

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Abstract

Objective: Down syndrome (DS) is the most common genetic cause of mental retardation, and affected individuals are more prone to infections, early senescence, Alzheimer's disease and other anomalies. One of the principal characteristic of this pathology is the stress oxidative. Individuals with DS have increased susceptibility to oxidative stress. The objective of this study is evaluated the degree of oxidative stress in an adult population.

Methods: 17 individuals with Down syndrome, aged from 16 to 37 years, with a mean age of 24.3 ± 4.3 years, participated in the study. Were analyzed several biochemical parameters (glucose, urea, creatinine, uric acid, total proteins, albumin, total bilirubin, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glutamic oxalacetic transaminase (GOT), glutamic-pyruvic Transaminase (GPT), Alkaline phosphatase, gamma GT, lactate dehydrogenase (LDH), ferritin, saturation transferrin, transferrin, iron, ferritin, vitamin B12 and folic acid); haematological parameters (red blood cells, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, mean platelet volume, leucocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils; selenium and zinc. The oxidation of proteins was analyzed by measuring the carbonyl groups in plasma; furthermore, it has measured the total antioxidant activity in plasma.

Results: There was statistically significant difference ($p < 0.05$) between the values total antioxidant activity of individuals with DS (1.08 ± 0.18 mM Trolox/L) and control groups (0.84 ± 0.15 mM Trolox/L). The levels of protein carbonyls in individuals with DS (0.51 ± 0.09 nM/ mg protein) were significant higher than the levels of protein carbonyls in control groups (0.34 ± 0.03 nM/ mg protein).

Conclusions: These results provide direct evidence that the oxidative stress is increased in individuals with DS. Since oxidative protein damage reflects increased degenerative processes, this data agrees with the reported premature aging, the increased incidence of cataracts and early Alzheimer changes in DS. This opens the possibility that antioxidant nutrient supplementation might help to ameliorate the pathology of DS.

Keywords: Internet addiction; Down syndrome; Oxidative stress; Carbonyl groups; Biochemical parameters; Hematological parameters; Total antioxidant activity

Abbreviations:

SD: Down Syndrome; GSH-Px: Glutathione Peroxidase; SOD: Superoxide Dismutase; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; GOT: Glutamic Oxaloacetic Transaminase; GPT: Glutamic-pyruvic transaminase; LDH: Lactate Dehydrogenase; EDTA: Ethylene Diaminetetra Acetic Acid; ml: Millilitres; μ l: Microlitres; HPLC: High Performance Liquid Chromatography; DNP: Dinitrophenylhydrazine; ELISA: Testing by enzyme-linked immunosorbent; IMC: Body Mass Index; TAS: Total Antioxidant Status; ABTS: Ácido 2,2'-azino-bis(3-ethylbenzthiazolin-6-sulfónico acid); SPSS: Statistical Package for the Social Sciences; SEEDO: Spanish society for the Studio of Obesity; mmol: Millimol; DNA: Deoxyribonucleic Acid; nM: Nanomol

Introduction

Down syndrome (DS), or trisomy 21, is a complex metabolic and genetic disorder that stems from the failure of chromosome 21 to segregate normally during meiosis [1].

Scientific evidence shows that individuals with Down syndrome (DS) have high levels of oxidative stress [2,3]. Oxidative stress is part of the fundamental biology of Down syndrome (DS). Trisomic cells are some sensitive to oxidative stress, suggesting oxidative imbalance in hydrogen peroxide metabolism, potentially contributing to accelerate aging observed in these persons [2]. Oxidative stress is defined as an imbalance between production of oxygen-derived free radicals and their removal by antioxidants. The presence of an extra chromosome 21 in DS results in overexpression of genes residing on that chromosome. This overexpression may contribute to a genetic imbalance in DS which propagates the development of ROS. Mitochondrial dysfunction is impacted by and also creates oxidative stress in DS [3]. The resulting gene dose effect is thought to account for most of the pathophysiology of SD [1]. Chromosome 21 contains several genes that have been implicated in oxidative stress related to neurodegeneration including Cu/Zn superoxide dismutase (SOD1),

amyloid precursor protein (APP), Ets-2 transcription factors, Down Syndrome Critical Region 1 (DSCR1) stress-inducible factor, beta-site APP cleaving enzyme (BACE) and S100 [3].

The oxidative modification of proteins by reactive species, especially reactive oxygen species, is implicated in the etiology or progression of several disorders and diseases. These reactive species form through a large number of physiological and non-physiological reactions. An increase in the rate of their production or a decrease in their rate of scavenging will increase the oxidative modification of cellular molecules, including proteins. The accumulation of modified proteins disrupts cellular function either by loss of catalytic and structural integrity or by interruption of regulatory pathways [4].

An excess of oxygen-derived free radicals could result in an extra demand for antioxidant nutrients like vitamins C and E, β -carotene, and selenium (cofactor for GSH-Px). Thus even normal serum concentrations of these nutrients could be functionally deficient in the face of excess demand. This opens the possibility that antioxidant nutrient supplementation might help to ameliorate the pathology of DS.

Our aim was to carry out the quantification the degree of oxidative stress through the analysis of several biomarkers of oxidative stress in individuals with DS.

Subjects and Methods

Subjects

Participants were 35 individuals with Down syndrome, aged from 16 to 37 years, with a mean age of 24.3 ± 4.3 years and a mean body mass index (BMI) of 29.8 ± 6.7 kg/m². The control group consists of a brother or sister of each one of the participants with DS of the study (Table 1).

	DS Subjects	Controls
Mean age, years (\pm SD)	24.3 ± 4.3	29.8 ± 6.7
Weigh (Kg)	73.5 ± 11.0	69.5 ± 8.7
Height (cm)	154.6 ± 6.5	1.68 ± 4.3
Body mass index, Kg/m ²	$30.7 \pm 4.1^*$	25.6 ± 4.9

Table 1: Characteristics of the individuals with Down Syndrome and control group; * Significant differences ($p \leq 0.05$) vs control group; Values expressed as mean \pm SD

Each participant or a legal guardian signed an informed consent form detailing the analyses and handling of the data. Both groups excluded individuals who took alcohol, tobacco, continuous medication, or vitamins. None of the individuals from any group reported any type of disease. The studies were performed according to the Principles of the Helsinki Declaration. The study was approved by the Ethics Committee of the University Catholic San Antonio.

Haematological and biochemical parameters

Biochemical parameters were determined in serum using an automated biochemical auto-analyser HITACHI Modular D β P (Roche Diagnostics, Switzerland). The parameters analysed were: glucose, urea, creatinine, uric acid, total proteins, albumin, total bilirubin,

cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glutamic oxalacetic transaminase (GOT), glutamic-pyruvic Transaminase (GPT), Alkaline phosphatase, gamma GT, lactate dehydrogenase (LDH), ferritin, saturation transferrin, transferrin, iron, ferritin, vitamin B12 and folic acid.

Haematological parameters were determined in EDTA-treated blood using an automated haematological analyser (Cell-Dyn 3700 and 4000, Abbott, IL, USA). The parameters analysed were: red blood cells, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, mean platelet volume, leucocytes, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Vitamin E, β -carotene and Vitamin C plasma analysis: Human plasma samples were spiked with concentrations ranging from 100 to 5000 μ g/ml for vitamin C and from 0.25 to 5 μ g/ml for vitamin E and β -carotene. Vitamin C in plasma was extracted as follows: plasma protein was precipitated with 60% methanol and 1mM EDTA. Plasma (100 μ l) was mixed with 400 μ l of 60% methanol/EDTA, incubated for 10min at 4°C before centrifuging at 12,000 rpm for 8 min. The clear phase was transferred to another polypropylene tube and evaporated to dryness under nitrogen. The dried extracts were dissolved in 100 μ l of methanol.

Vitamin E and β -carotene in plasma were extracted as follows: 100 μ l of plasma was deproteinized with 100 μ l of ethanol and was extracted with 600 μ l of chloroform. The extract was shaken for 5 min before centrifuging. The organic layer was extracted and evaporated to dryness under nitrogen. The dried extracts were dissolved in 100 μ l of methanol. All reconstituted antioxidants were mixed together before injecting into the HPLC system. Samples were quantified using peak area of vitamin C, vitamin E and β -carotene. Standard calibration curves were constructed by spiked drug-free pooled human plasma with a known amount of vitamin C, vitamin E and β -carotene. Vitamins C, E and β -carotene were separated on the LiChrospher 100 RP-18 column (125 \times 4 mm I.D.; particle size, 5 μ m) from Merck KGaA (Darmstadt, Germany), with a mobile phase of methanol-acetonitrile-tetrahydrofuran (75: 20: 5, v/v/v) at a flow-rate of 1.2 ml/min [5].

Zinc analysis: The serum determination of zinc was carried out according to a variant of Lewis' technique with slight changes [6]. We extracted 5 mL of venous blood without anticoagulant, that blood was heated in a bain-marie at 37°C for two hours so that the shrinking of the clot took place; after that, the blood was centrifuged at 2500 rpm for 10 minutes at room temperature and 1 mL of serum was extracted, we added deionised water to it until obtaining a 1:10 dilution, as in the case of control serum (seronormtm) and the standard solutions. The readings were carried out in a spectrophotometer of atomic absorption with a wavelength of 213 nm [6].

Selenium analysis: It has been established through atomic absorption spectrometry with a graphite camera and a Zeeman background editor. We have used palladium as a matrix modifier with a high level of sensitivity and little interference. The lamp is a discharge type without electrodes, with a 30 minutes warming-up. The wavelength used is 196.0 nm, the current intensity is 290 mA and the crack is 0.7 nm. The plasma is centrifuged at 500 g for 10 minutes. In order to set the standards we start from a 1 g/L certified pattern, diluted to a 10 mg/L concentration. The diluted standards are prepared in a matrix modifier 1/9 (v/v) with final concentrations of

0.63, 1.26 and 2.53 $\mu\text{mol/l}$. The target is prepared with ultras pure water and a modifier 1/9 (v/v) [7].

Antioxidant Activity

Total antioxidant Activity (TAS): Total antioxidant status (TAS) in plasma was measured by a chromogenic method (Randox Laboratories Crumham's, North Ireland). In this assay metmyoglobin reacts with hydrogen peroxide to form ferrylmyoglobin free radical species. Ferrylmyoglobin was incubated with the substrate (2,2'-amino-di[3-ethylbenzthiazole sulphonate]) and measured at 600 nm [8].

Protein Oxidation: Carbonyl groups Assay: Protein carbonyl content was measured by forming labelled protein hydrazones derivatives, using 2,4-dinitrophenylhydrazide (DNPH), which were then quantified spectrophotometrically [9]. Briefly, after precipitation of protein with an equal volume of 1% trichloroacetic acid (TCA), the pellet was re-suspended in 1 ml of DNPH 10 mM in 2N HCl. Separate blanks were prepared by adding 1 ml of 2N HCl without DNPH. Samples were left at room temperature for 1 h in the dark and vortexed every 15 min. An equal volume of 20% TCA was added and after centrifugation at 12000 g for 15 min at 4°C, pellets were washed three times with 1 ml of ethanol-ethylacetate mixture (1:1) to remove the free DNPH and lipids contaminants. The final pellet was dissolved in 1 ml of 6M urea and kept at 37°C for 1 h in a water bath with mixer. The solution was centrifuged for 15 min. at 12000 g. The carbonyl content was determined from the absorbance at 370 nm with the use of a molar absorption coefficient of 22000 mol/L [9].

Statistical analysis

Data are expressed as mean \pm SD. Statistical significance was evaluated by Student's t-test. Differences were considered significant at $p < 0.05$. All test statistical tests were performed with the SPSS statistical software, version 17.0 for Windows (SPSS Inc, Chicago, III).

Results

Biochemical and hematologic analysis

All the biochemical parameters analyzed are within the limits of normality (age-related reference values) and only significant differences ($p < 0.05$) were found in the uric acid values, higher in individuals with DS than in the control group (Table 2).

	DS Subjects	Controls
Serum albumin, g/dl	3.98 \pm 0.24	4.63 \pm 0.33
Uric acid mg/dl)	6.99 \pm 1.96*	4.70 \pm 1.65
Bilirrubine (mg/dl)	0.51 \pm 0.15	0.58 \pm 0.24
Iron ($\mu\text{g/dL}$)	91.87 \pm 41.91	84 \pm 21.32
Ferritin (ng/mL)	143.25 \pm 88.52	34 \pm 15
Tranferrina (mg/dl)	233.87 \pm 22.50	
Vitamin E ($\mu\text{g/mL}$)	11.03 \pm 5.51	10.91 \pm 5.01
Vitamin C ($\mu\text{mol/L}$)	24.57 \pm 8.78	25.26 \pm 7.04
-Carotene(γ A)	329.3 \pm 209.4	213 \pm 96.70
Folicacid(ng/mL)	8.87 \pm 12.77	5.88 \pm 2.02

Vitamin B12 (pg/mL)	419.7 \pm 189.80	555.20 \pm 88.66
Copper($\mu\text{g/dL}$)	107.86 \pm 10.51	112.20 \pm 16.49
Cinc ($\mu\text{g/dL}$)	73.06 \pm 10.31	84.40 \pm 15.14
Seleniun($\mu\text{g/mL}$)	83.56 \pm 18.14	79.33 \pm 7.99

Table 2: Biochemical parameters in individuals with Down syndrome and control group; * Significant differences ($p \leq 0.05$) vs control group

Regarding the plasma values of iron, zinc, selenium, vitamin E, vitamin B12, vitamin C and β -carotene we did not observe any significant differences when comparing individuals with DS and individuals from the control group.

Antioxidant Status

We found statistically significant differences ($p < 0.05$) between the values of the total antioxidant activity (TAS) in DS patients (1.08 \pm 0.34 mM Trolox/L) and the control groups (0.84 \pm 0.15 mM Trolox/L) (Figure 1), and also levels of carbonyl protein, in individuals with DS (0.51 \pm 0.24 nM/mg protein), were significant higher than the levels of carbonyl protein in control groups (0.34 \pm 0.13 nM/mg protein) (Figure 2).

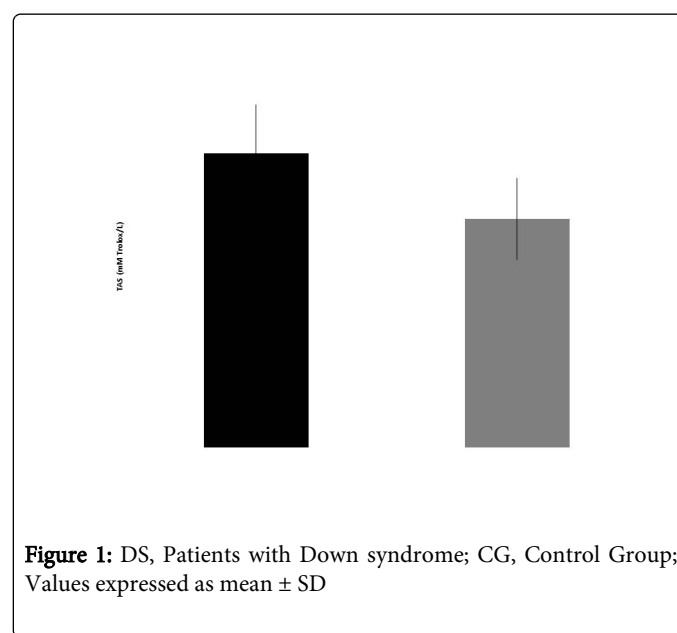


Figure 1: DS, Patients with Down syndrome; CG, Control Group; Values expressed as mean \pm SD

Discussion

Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. This ratio can be altered by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), or a decrease in antioxidant defense mechanisms [10]. Oxidative stress is implicated in DS pathogenesis and progression due to a deregulation of gene/protein expression associated with the trisomy of DS. We found in the uric acid values, higher in individuals with DS than in the control group. Other authors [11-13] coincide with us, and reported uric acid values, higher in individuals with DS than in the control group. The uric acid is an efficient hydrophilic plasma antioxidant that may be associated with the increased resistance of serum lipids to oxidation which play an important role in

the atherogenic process [14]; but the origin of this biochemical anomaly is not clear. Some authors attribute this increase to alterations in glomerular filtration [15], but the formation of uric acid can also occur through the xanthine oxidase with a concomitant production of superoxide.

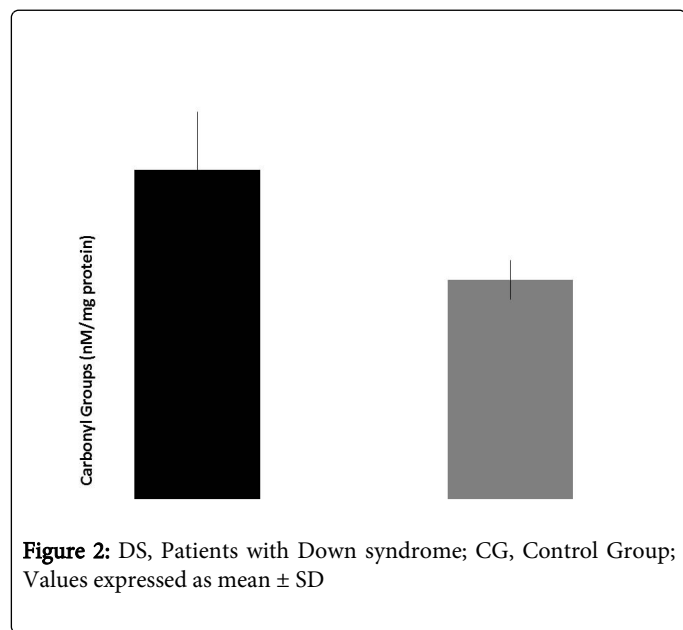


Figure 2: DS, Patients with Down syndrome; CG, Control Group; Values expressed as mean ± SD

Regarding the plasma values of iron, zinc, selenium, vit E, vit B12, vit C and β -carotene we did not observe any significant differences when comparing individuals with DS and individuals from the control group. In the same way, Garlet et al. [16], found out that the average levels of vitamin E showed no significant differences between DS individuals compared to controls. However, Pallardó et al. [11] reported that the concentrations of vitamin C were increased in young DS patients, but not in older patients in comparison with the control group in the same age range. They also reported that the levels of vitamin E in DS patients did not differ from the values determined in donors from the control group. Some reports suggest that levels of vitamin B12 serum are reduced for those individuals with Down syndrome [17] while other reports have not found that [18].

Barden [20] shows significantly higher values of vitamin A in individuals with Down syndrome and not any differences in the carotene values.

Down syndrome patients have often been found elevated levels of copper in erythrocytes, neutrophils, platelets, and serum [17]. Several reports suggest that Down patients have plasma levels of zinc below normality [19-21]. In addition, it has been found that DS children become deficient in a zinc-containing insulin-like growth factor type 1 (IGF-1) after one year of age [22]. Kadrobova et al. [23] pointed that some adaptations to permanent oxidative stress and changed biochemical functions in DS individuals may lead to increased requirements of zinc; this means that it is very likely that DS people have an increased need for zinc as they use a larger percentage of it, available for manufacturing the SOD-1. It also alludes to the effects of increased SOD-1 activity. However, other authors [24] reported that the blood levels of zinc were normal, coinciding with our results.

Some authors reported that Down patients may have the plasma levels of selenium below normality [25].

Ani et al. [26] reported that an excess of oxygen derived free radicals could result in an extra demand for antioxidant nutrients like vitamins C and E, carotenes, zinc and selenium (cofactor for GSH-Px). Thus, even normal serum concentrations of these nutrients could be functionally deficient in the face of an excessive demand.

Carratelli et al. [27] coincide with us and found that the reactive oxygen species and the total antioxidant capacity were significantly higher in children with DS than in a control group. In the same way there are several authors [28,29] who found a higher level of carbonyl protein which also coincides to our results. These results agree with the presumption of an increased oxidative stress in individuals with DS. Cu, Zn-SOD levels are high and excessive Cu, Zn-SOD gene dosage can lead to the increased production of H_2O_2 , which is a toxic oxidant. Hydrogen peroxide initiates Fenton-type reactions and yields very reactive hydroxyl radicals leading to an increased damage of plasma proteins. Oxidative damaged proteins can act as prooxidants, leading to the damage of additional macromolecules.

An increase of the carbonyl compounds that are biomarkers of oxidative stress has appeared in DS and Alzheimer's Disease brains. This seems to be the result of a multifactorial process. Carbonyls, which are cytotoxic metabolic intermediates, are detoxified by either oxidation catalyzed by aldehyde dehydrogenase (ALDH), or by reduction to their corresponding alcohol by carbonyl reductase and/or alcohol dehydrogenase. An ALDH deficiency has been detected in both disorders and may be involved in the pathogenesis of both diseases [15,30,31].

Despite the fact that we did not notice a deficiency of vitamins and antioxidant minerals and the increased plasmatic levels of uric acid in DS, the antioxidant protection of children with DS was not enough to prevent protein oxidation.

Conclusions

We only reported significant differences when comparing the biochemical parameters in individuals with DS and controls in the uric acid values, higher in DS individuals. The highest mean value of the oxidative stress biomarker shows an increased rate of oxidative damage in individuals with DS. Since oxidative protein damage reflects increased degenerative processes, this data agrees with the reported premature aging, the increased incidence of cataracts and early Alzheimer changes in DS.

An excess of oxygen-derived free radicals could result in an extra demand for antioxidant nutrients like vitamins C, E, β -carotene, selenium and zinc (cofactor for GSH-Px). Thus even normal serum concentrations of these nutrients could be functionally deficient in the face of excessive demand. This opens the possibility that antioxidant nutrient supplementation might help to ameliorate the pathology of DS.

Further studies are needed to establish the potential of the antioxidant supplementation in children with DS to prevent oxidative damage of proteins and other biomolecules.

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