EVALUATION OF AGE-RELATED LEVELS OF BRAIN-DERIVED NEUROTROPHIC FACTOR, MALONDIALDEHYDE AND GLUTATHIONE PEROXIDASE IN DOWN SYNDROME PATIENTS

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ABSTRACT

Background: *As a result of the substantial rise in the life expectancy of people with Down syndrome in recent years, there is now a greater group of individuals with the condition who show early signs of ageing. The current study was carried out to evaluate age-related changes in the blood levels of a predictive biomarker for early identification of neurodegenerative alterations in people with Down syndrome.*

Methods: *This study was conducted on two groups of Down syndrome patients: group 1 consisted of 30 children between the ages of 2 and 7years of age; group 2 consisted of 30 adolescents between the ages of 17 and 20 years of age. The control group was composed of 60 healthy children and adolescents who were matched for age and sex. All the cases and controls were subjected to: Complete history taking, Clinical examination, Anthropometric measurements, Karyotype analysis and Blood sampling to assess the serum level of Brain-derived neurotrophic factor, Malondialdehyde and Glutathione peroxidase.*

Results: *Serum levels of Brain-derived neurotrophic factor and Malondialdehyde revealed a statistically significant increase ($p<0.05$), while Glutathione peroxidase showed a statistically significant decrease in between the Down syndrome groups 1 and 2 (aged 2 to 7 years and 17 to 20 years, respectively) when compared to the control group. Furthermore, there were a highly significant increase ($P<0.05$) in only Brain-derived neurotrophic factor and Malondialdehyde levels between the two Down syndrome groups.*

Conclusion: *we conclude that based on the data that is now available we are unable to prove the dependability of the current biomarkers in the diagnosis of neurodegenerative disorders in Down syndrome.*

Keywords: *Biomarkers, Down's syndrome, Brain-derived neurotrophic factor, Malondialdehyde and Glutathione peroxidase.*

BACKGROUND

Down syndrome (DS), also known as trisomy 21, is characterized by a number of dysmorphic features, such as a space between the first and second toes; the sandal gap deformity; tiny ears; upward-slanted eyes; and a protruding large tongue **(Hulten et al., 2008)**. Alzheimer dementia (AD) and the most prevalent neurological or psychiatric condition associated with ageing that affects DS individuals are comparable **(Lott and head, 2019)**. Typically, memory, learning, and orientation deterioration are the first signs, which are commonly followed by an increase in reliance **(Lisa and Rikus, 2004)**. DS has a better prognosis than other trisomies since chromosome 21 is the smallest human chromosome and only contains 300–400 genes **(Aït et al., 2007)**. Therefore, improving the patients' quality of life is essential.

Because these individuals have a pre-existing and variable intellectual handicap that might conceal a gradual decline in cognitive performance, clinical identification of dementia and other neuropathological disorders by cognitive testing is challenging in these people. Although accurate, positron emission tomography (PET) and cerebrospinal fluid (CSF) biomarkers are expensive, invasive, and particularly challenging in such a vulnerable group. The importance of blood biomarkers as a valuable and practical tool for diagnosing AD has been highlighted as a result of improvements in sensitive detection methods **(Montoliu-Gaya et al., 2021)**.

The discovery that a biomarker's existence or change can foretell whether an individual or group of persons would be more likely to suffer a positive or negative reaction to exposure to a medication or environmental contaminant defines a biomarker as predictive **(FDA-NIH working group, 2016)**.

A collection of biomarkers called neurotrophic factors (NFTs) has drawn a lot of interest in the study of Alzheimer's disease. These tiny proteins primarily affect neuronal survival, axonal

guidance, and memory cognition **(Schindowski et al., 2008)**. The most widely distributed neurotrophin in the central nervous system, brain-derived neurotrophic factor (BDNF), is involved in neurogenesis, neuroplasticity, neuronal development, and neuronal survival. Patients with AD or moderate cognitive impairment had lower amounts of BDNF, pro-BDNF, and BDNF mRNA in their brains, according to postmortem investigations. Reduced BDNF production has also been proposed as one explanation of age-related cognitive deterioration **(Arslan et al., 2021)**.

Flores-Aguilar et al., revealed that neuroinflammatory markers in the brains of people with DS (including BDNF) diminish as people age, maybe due to cell depletion and degeneration **(Flores-Aguilar et al., 2020)**.

A pathogenic mechanism for cell ageing, and neurologic problems in DS has been proposed: oxidative stress **(He et al., 2016)**. The SOD1 gene is located on chromosome 21 (21q22.11), so overproduction of SOD1 in DS results in excess generation of hydrogen peroxide (H2O2), which disintegrates to hydroxyl radicals that target polyunsaturated fatty acids in cell membrane phospholipids, giving rise to lipid peroxidation that has been observed in the brain of DS patients and may be the cause **(Ikeda et al., 2009)**.

Children with DS have fibroblasts that have a higher ratio of SOD1/Glutathione Peroxidase (GPx) activity, producing a lot of H2O2 and inducing intracellular oxidative stress. Cellular senescence in DS is caused by a decline in the antioxidant defense system, mitochondrial malfunction, and ageing. As AD neuropathology develops, cognitive impairment is further exacerbated **(De Haan et al., 1996)**.

Glutathione peroxidase, one of the most significant antioxidant enzymes, is a selenium protein that is essential for preventing peroxide accumulation in cells and, by preventing the formation of lipid peroxide, may play a special protective role in DS children's cells against the progression of neurobiological abnormalities **(Garcez et al., 2005)**.

The early diagnosis and treatment of AD may assist to delay the functional deterioration usually associated with these conditions, therefore research into AD in people with DS is still underway. According to studies,

the life expectancy of DS has significantly increased, from 12 years in 1949 to around 60 years now **(Esbensen, 2010)**. A bigger proportion of persons with DS are showing early age-related changes in their health as a result of this extended life expectancy. In order to aid in the early diagnosis of neurodegenerative changes in those individuals, this study evaluated age-related changes in blood levels of BDNF, malondialdehyde, and glutathione peroxidase.

Ethical Consideration:

- Approvals from the Pediatric Department and Ethics Committee, Faculty of Medicine, Ain Shams University were obtained.
- This work was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. The patients' legal guardians were subjected to an informed consent before enrollment in the study.
- The data of the study is confidential and the patient or caregiver has the right to keep it.
- The Patient has the right to withdraw from the study at any time.
- No conflict of interest in regards of the study or publication.
- There was no funding for the study or publication from any source.

Inclusion Criteria:

Any patient with confirmed dowen syndrome clinically and by karyotyping of sex and aging from 2-7years or 17-20years.

Exclusion Criteria:

Any patient who was complaining or diagnosed with any other chronic illnesses (affecting the brain, heart, lung, liver, and kidney), neurodegenerative disorders, and/or errors of metabolism.

Sample Size:

According to Epi info 2000, the sample size was computed with a precision of (2%), a 95% confidence interval, and a sample size of 60 children and adolescents with Down's syndrome and 60 healthy controls. 85% of the study power was used in the analysis. Convenient samples composed in sample type. (CDC Epi info V7.0.8.3).

Methods:

This is a comparative case control study that was carried out in the outpatient clinic for children with special needs at the National

Center for Research and Children's Hospital, Ain Shams University, during the period from September 2020 to September 2022. Our cases were selected by a simple random method and the cases were categorized as following:

- The first group consisted of 30 children aged 2-7 years assigned as group 1 (8 males and 22 females).
- The second group consisted of 30 adolescents aged 17-20 years assigned as group 2 (15 males and 15 females).
- The control group composed of 60 seemingly healthy children and adolescents who were matched for age and sex (30 children aged 2-7 years and 30 adolescents aged 17-20 years).

All the included cases and controls were subjected to the following:

- A thorough history is taken, which includes antenatal, natal, postnatal, and family histories.
- Clinical examination with emphasis on Anthropometric measurements in the form of weight, height, and head circumference, dysmorphic features and systemic examination including chest, heart, abdomen and neurological assessment.
- Intelligence Quotient Test: Stanford-Binet Intelligence Scale, Fourth Edition (SBIV) for all the studied cases **(Couzens et al., 2011)**.
- Karyotype analysis **(Speicher et al., 1996)**.
- A 5 ml sample of whole blood was taken, and the blood was allowed to clot by being left at room temperature unattended for 15–30 minutes. After that, we extracted the clot by centrifuging in a chilled centrifuge for 10 minutes at 1,000–2,000 x g. The resultant supernatant, called serum, was then used for the determination of malondialdehyde (ELISA Kit ab287797) as a marker of lipid peroxidation, glutathione peroxidase (ELISA Kit ab193767), and brain-derived neurotrophic factor (ELISA Kit ab212166).

Statistical Analysis:

The Statistical Package for Social Science (SPSS) 25 was used to code, and tabulate the obtained data. Using the proper tests, descriptive and analytical statistics were both carried out.

RESULTS

Our results will be demonstrated in the following tables:

Table (1): Comparison of descriptive data between control & DS group 1

Variables	Control group	DS group 1	p value	t test
	2-7 years (n= 30)	**2-7 years (n= 30)**	-	-
Age (years)	4.03 ± 1.61	4.48 ± 1.64	-	-
Gender (M/F)	18/12 (60%/40%)	15/15 (50%/50%)	-	-
Weight (kg)	17 ± 0.28	15± 0.61	p value 0.001*	t value 16.321
Height (cm)	105± 0.46	102± 0.35	p value 0.001*	t value 28.428
HC(cm)	50± 0.31	49± 0.46	p value 0.001*	t value 9.874

Data were expressed as mean ± SD. n= Number *p< 0.01= highly significant
M/F= male/female. HC=Head circumference. n= Number

Table 1 shows that the mean values of weight, height and head circumference are significantly lower in DS group 1 when compared to control group. Moreover, the high t value between the two groups confirms the significance.

Table (2): Comparison of descriptive data between control & DS group 2

Variables	Control group	DS group	p value	t test
	17-20 years (n= 30)	**17-20 years (n= 30)**	-	-
Age (years)	18.53 ± 1.20	18.87 ± 1.27	-	-
Gender (M/F)	15/15 (50%/50%)	22/8 (73.3% /26.7%)	-	-
Weight (kg)	62± 0.37	69.6± 0.09	p value 0.001*	t value 109.32
Height (cm)	174± 0.38	165± 0.08	p value 0.001*	t value 126.94
HC(cm)	55± 0.50	53± 0.39	p value 0.001*	t value 17.275

Data were expressed as mean ± SD. n= Number
M/F= male/female. HC=Head circumference. n= Number
*p< 0.01= highly significant.

Table 2 shows that the mean values of weight, height and head circumference are significantly lower in DS group 2 when compared to control group. Moreover, the hight value between the two groups confirms the significance. The t values in table 2 are more than table 1 which can be explained by the findings and symptoms are clearer with advanced age.

Table (3): Cytogenetic analysis in both DS groups

VARIABLES	DS GROUP 1 (N= 30)	DS GROUP 2 (N= 30)	P-VALUE
Non Disjunction	93.3%	90%	0>.001(insignificant)
Translocation	3.3%	6.6%	0<.001 (significant)
Mosaic	3.3%	3.3%	0>.001(insignificant)

Table 3 shows almost same values in both groups in terms of cytogenetic analysis results, with significant difference between Both groups regarding translocation genotype.

Table (4): Comparison between the mean values of different studied biomarkers between the control and DS group 1

Variables	Control group (n= 30)	DS group 1 (n= 30)	t test	P value
BDNF (pg/ml)	9.57 ± 6.04	17.18 ± 8.87	-3.882	0.001*
MDA (μmol/L)	0.72 ± 0.26	1.18 ± 0.42	-5.097	0.001*
GP_x(U/gHb)	23.26 ± 5.61	16.98 ± 5.09	4.540	0.001*

Data were expressed as mean ± SD. n= Number
*p< 0.01= highly significant. BDNF: Brain Derived neurotrophic Factor
MDA: Malondialdehyde, GPx: Glutathione peroxidase

Table 4 shows a highly statistically significant increase in DS group 1 than control group as regards BDNF, Malondialdehyde while a significant decrease in the Glutathione peroxidase.

Table (5): Comparison between the mean values of different studied biomarkers between the control and DS group 2

Variables	Control group (n= 30)	DS group 2 (n= 30)	t test	P value
BDNF (pg/ml)	11.14 ± 4.82	25.85 ± 8.14	-8.514	0.001*
MDA (µmol/L)	0.85 ± 0.28	1.61 ± 0.65	-5.868	0.001*
GP_x (U/gHb)	26.54 ± 6.36	18.39 ± 5.29	5.396	0.001*

Data were expressed as mean ± SD. n= Number
*p< 0.01= highly significant. BDNF: Brain Derived neurotrophic Factor
MDA: Malondialdehyde, GPx: Glutathione peroxidase

Table 5 shows a highly statistically significant increase in DS group 2 than control as regards BDNF, Malondialdehyde while a significant decrease in the Glutathione peroxidase.

Table (6): Comparison between the mean values of different studied biomarkers between the DS group 1 and group 2

Variables	DS group 1 (n= 30)	DS group 2 (n= 30)	t test	P value
BDNF (pg/ml)	17.18± 8.87	25.85± 8.14	-3.945	0.001*
MDA (µmol/L)	1.18 ± 0.42	1.61 ± 0.65	-3.025	0.004*
GPx (U/gHb)	16.98 ± 5.09	18.39 ± 5.29	-1.049	0.299 ^{NS}

Data were expressed as mean ± SD. n= Number
N.S= Not Significant = p> 0.05. *p< 0.01= highly significant. BDNF: Brain Derived neurotrophic Factor, MDA: Malondialdehyde, GPx: Glutathione peroxidase

Table 6 shows a highly statistically significant increase between both DS groups 1 and 2 as regards BDNF and Malondialdehyde.

DISCUSSION

Down syndrome is one of the most common genetic causes of learning disabilities and ¬¬congenital malformations in the human population. It occurs with a prevalence of 1 in 700 live births in the United States and results from partial or complete triplication of chromosome 21 **(Mai et al., 2019)**. It is characterized by accelerated ageing and is linked to the early onset of dementia. The average lifespan of DS has greatly risen as a consequence of advancements in health and social care **(Day et al., 2005)**. By the age of 40, virtually all patients with DS develop an Alzheimer disease-like neuropathology **(Teipel and Hampel, 2006)**. In our study, we

evaluated age-related changes in serum levels of BDNF, malondialdehyde and glutathione peroxidase for early detection of neurodegenerative changes in DS patients.

In the current study, almost all the studied anthropometric parameters showed a significant decrease in our cases compared to controls and this goes in concordance with **Sachdev et al. (1981)** may be due to the retarded growth potential in DS patients. In our study, cytogenetic analysis revealed 93.3% non-disjunction, 3.3% translocation, and 3.3% mosaic in 30 DS aged 2-7 years. However, there was 90% non-disjunction, 6.6% translocation, and 3.3% mosaic in 30 DS aged 17-20 years. Our results were in accordance with the results of **Verma et al. (1991)**, in which the karyotype analysis results were: 93.3% non-disjunction, 4.1% translocation, and 2.6% mosaics. However, in **Mokhtar et al. (2003)**, the results were: non-disjunction 95.4%; translocations, 2.7%; and mosaics 0.7%.

BDNF is a neuropeptide that has many important functions in synaptic development and plasticity **(Wang et al., 2022)**. In our study, there was an increased plasma level of BDNF in DS groups compared to control groups (p value < 0.001), and there was a highly significant age-related increase in the DS group 2 (17-20 years) compared to the DS group 1 (2-7 years). These results were consistent with those of **Dogliotti et al. (2010)**, and **Tarani et al. (2020)**, in which there was a substantial age-related rise in plasma BDNF in DS patients compared to controls. A high circulating BDNF concentration may shield DS patients from the clinical side effects of ageing. However, the aging-related decline in peripheral BDNF levels may make DS patients more susceptible to dementia clinical symptoms **Dogliotti et al., 2010**.

Malondialdehyde (MDA), is widely assessed as a marker of lipid peroxidation and oxidative stress in vivo since it is a primary end product of the oxidation of polyunsaturated FA. In our study, there was a significant increase in serum MDA levels in DS groups compared to control groups ($p< 0.001$), denoting an increased rate of oxidative damage in individuals with DS. These results agreed with those of **Ikeda et al., 2009** who reported elevated MDA concentrations in the serum of DS patients. Also, one of the effects of an imbalance in the activity of the antioxidant enzymes, according to **Jovanovic et al., 1998** was elevated MDA levels in the urine

of DS patients. However, our results do not agree with the **Muchova et al. 2001** study in which the level of the lipid per-oxidation product, serum MDA, did not reveal a significant difference in MDA between the DS and control groups. Additionally, in accordance with **Casado et al. (2007)**, we discovered that MDA levels were considerably higher in DS groups than in control groups, although there was no discernible difference between MDA levels between DS groups and control groups according to age categories. Our results were also in agreement with **Garaiova et al. (2004)**, study in which there was an increased level of MDA in persons with DS which supports the hypothesis of premature ageing in persons with DS over the age of 25 years. In regards of glutathione peroxidase levels in our study we demonstrated a statistically significant decrease in group 1 and 2 DS when compared with normal controls, moreover a decline in the GPx values as the DS patients gets older. These findings accord with **Parisotto et al. 2015** who stated that the glutathione system is affected in DS The levels of glutathione are decreased in these individuals and **Pastore et al. 2003** who mentioned that there is a systemic decrease of all glutathione forms, including glutathionyl-hemoglobin, in the blood of children with Down syndrome.

CONCLUSIONS

- A significantly elevated serum BDNF with age was detected in our study, this could be a defense mechanism to stop cognitive decline, memory issues, and behavioral deterioration in children of this age.
- Additionally, oxidative stress, especially from lipid peroxidation, and disturbed antioxidant status in DS patients were increased by age and could be considered one of the mechanisms involved in neurodegenerative disorders and the early occurrence of AD in those patients.

Recommendation

- Further longitudinal research is required for the early detection of age-related health changes in DS patients and the development of early intervention policies to promote healthy aging for this group of patients.
- CSF accurately reflects changes in the central nervous system or developing neuropathology.

So, we recommend the study of these biomarkers in C.S.F.

Study limitation:

One of the limitations of this study was that the age group studied did not clearly give us a chance to observe the change in serum levels that might occur at later ages for these biomarkers.

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