Thiol Disulfide Balance Oxidative Stress and Paraoxonase 1 Activities in Children and Adolescents Aged 6-16 Years with Specific Learning Disorders

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Citation: Savas HB, Sayar E, Kara T. Thiol Disulfide Balance Oxidative Stress and Paraoxonase 1 Activities in Children and Adolescents Aged 6-16 Years with Specific Learning Disorders. Electron J Gen Med. 2021;18(3):em290. https://doi.org/10.29333/ejgm/10837

INTRODUCTION

Specific learning disorders (SLD) are some of the most common neurodevelopmental disorders. They refer to problems that continue in one of three areas: reading, writing and mathematics, and they form the basis of one’s learning ability. SLD is a neurodevelopmental disorder that comes to the forefront in children at school age. The prevalence of SLD in school-aged children was reported to be in the range of 5-15% [1-3]. SLD has a multifactorial etiology including environmental factors, neuropsychological factors and genetic factors [4]. Studies have shown that children with learning difficulties are exposed to toxic metals, especially Pb, Al and Ni. Additionally, most of these children had low performance scores in cognitive ability tests in comparison to their healthy peers [5]. It was reported that zinc deficiency in childhood may cause delays in mental and physical development and lead to formation of learning difficulties [6]. It is known that memory and learning function is at stake because hippocampus and frontal cortex are injured by copper directly or through oxidative stress [7]. It is known that the role of oxidative stress in toxicological mechanisms due to metal exposure is one of the most significant mechanisms [5]. In animal experiments, it was found that oxidative damage in the cerebral cortex and hippocampus may contribute to a lack of cognitive functions and learning [8]. On the other hand, there is not any research in the literature, that evaluates some oxidative stress parameters and learning [8]. On the other hand, there is not any research in the literature, that evaluates some oxidative stress parameters in children and their relationship with SLD. Our aim of study is to determine the potential relationship between children with SLD and zinc, copper, paraoxonase 1, arylesterase, ischemia-modified albumin, thiol balance, oxidative stress index, total antioxidant status, total oxidant status, and routine biochemical parameters.

METHODS

Study Design

The study involved 44 children and adolescents aged between 6 and 16. All participants in the case group (n=22) had a diagnosis of a specific learning disorder based on psychometric tests and psychiatric evaluation. Patients with psychiatric diseases other than SLD and those with any organic disease were excluded from the study. All participants were assessed with the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children. Total oxidant status, total antioxidant status, thiol balance, oxidative stress index, 25 OH Vitamin D, reduced thiol status, native thiols and oxidized thiols were measured by spectrophotometric methods.
Results of the scale, five separate combined scores are obtained, namely the Verbal Concept Converted Score (VCCS), Perceptual Reason Converted Score (PRCS), Working Memory Transformed Score (VMTS), Transaction Speed Transformed Score (TSTS) and All Concept Converted Score (VCCS), Perceptual Reason Converted Score (PRCS), Working Memory Transformed Score (VMTS), Transaction Speed Transformed Score (TSTS) and All Scale Intelligence Score (ASIS). The scale was adapted to Turkish in 2011 by Uluç et al. [18].

Statistical Analysis

categorical variables were assigned with calculated frequencies and percentages, and the continuous ones with mean, standard deviation and median values. The appropriate method for identification of the normal distribution of the continuous variables was Kolmogorov Smirnov test. Significance level was statistically defined as p < 0.05. Inter-group comparative analysis of the laboratory results was achieved with ANOVA. Number Cruncher Statistical System, NCSS 11 (2016) was a statistical analytical package program that we utilized in our analysis.

Results

Distribution of SLD patients by types were reading disorder (RD): 4, arithmetic disorder (AD): 2, spelling disorder (SD): 3, combined learning disorders (CD): 13. the scores of WISC-IV of the participants were verbal comprehension: mean + SD: 91.16 + 13.84; perceptual reasoning: mean + SD: 90.41 + 11.47; working memory: mean + SD: 93.25 + 6.28; processing speed: mean + SD: 90.92 + 12.72; total scale score mean + SD: 88.58 + 9.56.

Compared to the control, the SLD group had statistically significantly lower values for PON1 antioxidant enzyme activity (p = 0.045), ARY antioxidant enzyme activity (p = 0.001), 25 OH Vitamin D (p = 0.005), reduced thiol ratio (p = 0.028), thiol oxidation reduction rate (p = 0.01), native thiols (p = 0.024) and significantly higher values for total oxidant status (p = 0.017), oxidized thiol ratio (p = 0.028). Both were indifferent based on the statistical significance level (p > 0.05) for the other parameters. Table 1 presents the sociodemographic data of children diagnosed with specific learning disorders. The detailed results of the comparison between the groups are given in Table 2. ROC Curve of statistically significant laboratory parameters were given in Figure 1. Area under the ROC Curve for statistically significant laboratory parameters were given in Table 3.

ROC (Receiver Operator Characteristics Curve) analysis and graph:

The important thing in evaluating ROC curves is the area under the curve (AUC-Area Under The Curve). The closer this area gets to 1, the success of the model for using the relevant laboratory parameter in the diagnosis of the disease increases. The more hyperbolic the ROC analysis curve of a parameter and the greater the angle with the x-axis, the more meaningful it becomes.
Table 1. Sociodemographic Data of Children Diagnosed with Specific Learning Disorders

| Parameters                  | Distribution                               |
|-----------------------------|--------------------------------------------|
| Sex                         | Male: 14 (63.64%) / Female: 8 (36.36%)     |
| Age                         | 8.41±1.56                                  |
| Number of siblings          | 2.77 ±1.11                                 |
| Sibling order               | 2.09 ±1.23                                 |
| Dominant hand               | Right: 17 (77.27%) / Left: 5 (22.73%)      |
| Dominant foot               | Right: 16 (72.73%) / Left: 6 (27.27%)      |
| Dominant eye                | Right: 15 (68.18%) / Left: 7 (31.82%)      |
| Severity of Specific Disability | Mild: 3 (13.64%) / Moderate: 11 (50%) / Severe: 8 (36.36%) |
| Specific Learning Disability in Family | Yes: 10 (45.45%) / No: 12 (54.55%) |
| Speech delay in the child   | Yes: 12 (54.55%) / No: 10 (45.45%)         |
| Speech delay in family      | Yes: 11 (50%) / No: 11 (50%)               |
| Mother’s age                | 35.64±5.19                                 |
| Mother’s occupation         | Not employed: 21 (95.45%) / Corporate: 1 (4.55%) |
| Mother’s education          | Literate: 1 (4.55%) / Primary education: 19 (86.36%) / University and higher: 2 (9.09%) |
| Father’s age                | 39.59±6.58                                 |
| Father’s occupation         | Corporate: 2 (9.09%) / Freelance: 20 (90.91%) |
| Father’s education          | Literate: 1 (4.55%) / Primary education: 18 (81.82%) / University and higher: 3 (13.63%) |
| Monthly income status       | 0-2 thousand; 3 (13.64%), 2-4 thousand; 16 (72.72%), 4-6 thousand; 2 (9.09%), 6 thousand and higher; 1 (4.55%) |

The sociodemographic data of the 22 children at the ages of 6-16 with specific learning disorders are shown in the table.

Table 2. Evaluation of Biochemical Parameters in Children with Specific Learning Disorders

| Parameters                             | Unit               | Control Group (n=22) mean±SD | Specific Learning Disorders Group (n=22) mean±SD | P   |
|----------------------------------------|-------------------|------------------------------|-----------------------------------------------|-----|
| Lymphocytes                            | cells per microliter of blood | 3250.91±1421.43              | 2993.64±1793.60                              | 0.463|
| Neutrophils                            | cells per microliter of blood | 3345.91±1141.43              | 3885.91±1529.92                              | 0.192|
| Neutrophils/Lymphocytes                | Ratio             | 1.27±0.72                    | 1.38±0.67                                    | 0.581|
| Leukocytes                             | cells per microliter of blood | 7515.91±1455.85              | 7888.18±1918.26                              | 0.472|
| Vitamin B12                            | µg/ml             | 465.86±128.93                | 434.36±179.23                                | 0.507|
| 25 OH Vitamin D                        | ng/ml             | 24.36±6.69                   | 18.64±6.18 *                                 | 0.005|
| Paraoxonase 1                          | U/l               | 665.05±101.60                | 607.23±82.77 *                               | 0.045|
| Arylesterase                           | U/l               | 824.41±91.61                 | 667.82±73.99 *                               | <0.001|
| Total Antioxidant Status               | mmol Trolox equivalent/l | 1.35±0.23                   | 1.34±0.21                                    | 0.874|
| Total Oxidant Status                   | µmol H₂O₂ equivalent/l | 3.10±1.46                   | 3.94±0.63 *                                  | 0.017|
| Oxidative Stress Index                 | Ratio             | 2.41±1.37                    | 3.00±0.68                                    | 0.077|
| Total Thiol                            | µmol/l            | 337.11±22.39                 | 330.17±47.01                                 | 0.536|
| Native Thiol                           | µmol/l            | 280.49±27.15                 | 257.13±38.22 *                               | 0.024|
| Disulfide                              | µmol/l            | 28.30±15.43                  | 36.52±14.32                                  | 0.074|
| Reduced Thiol Ratio                    | Ratio             | 83.45±8.47                   | 78.09±7.07 *                                 | 0.028|
| Oxidized Thiol Ratio                   | Ratio             | 8.27±4.23                    | 10.96±3.53 *                                 | 0.028|
| Thiol Oxidation Reduction Ratio        | Ratio             | 1295.28±636.68               | 844.93±462.89 *                              | 0.01|
| Ischemia modified albumin              | g/l               | 1.03±0.22                    | 1.07±0.14                                    | 0.447|
| Zinc                                   | µg/dl             | 84.56±42.95                  | 99.89±41.09                                  | 0.233|
| Copper                                  | µg/dl             | 107.52±30.64                 | 113.76±26.31                                 | 0.472|

* The Specific Learning Disorders group was statistically significantly different from the control (p <0.05). Ratios: Oxidative Stress Index = Total Oxidant Status / Total Antioxidant Status. Reduced Thiol = (Native Thiol / Total Thiol) * 100. Oxidized Thiol = (Disulfide / Total Thiol) * 100. Thiol Oxidation Reduction Ratio = (Native Thiol / Disulfide) * 100

Table 3. Area Under the ROC Curve for Statistically Significant Parameters

| Test Result Variable(s) | Area | Std. Error* | Asymptotic Sig. | Asymptotic 95% Confidence Interval |
|-------------------------|------|-------------|-----------------|------------------------------------|
|                         |      |             |                 | Lower Bound | Upper Bound |
| 25 OH Vitamin D (ng/ml) | .262 | .075        | .007            | .116       | .409        |
| Paraoxonase 1 (U/l)     | .301 | .086        | .024            | .131       | .470        |
| Arylesterase (U/l)      | .133 | .064        | .000            | .008       | .258        |
| Total Oxidant Status (µmol H₂O₂ equivalent/l) | .692 | .086 | .029 | .523 | .861 |
| Native Thiol (µmol/l)   | .310 | .080        | .031            | .152       | .468        |
| Reduced Thiol (Ratio)   | .298 | .083        | .021            | .134       | .461        |
| Oxidized Thiol (Ratio)  | .699 | .083        | .024            | .537       | .862        |
| Thiol Oxid. Red. Ratio (Ratio) | .298 | .083 | .021 | .134 | .461 |

The test result variable(s): 25 OH Vitamin D, Paraoxonase 1, Arylesterase, Reduced Thiol, Oxidized Thiol has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5
The incidence of SLD in childhood was found to be in the range of 5-15% [3]. To date, sufficient molecular and biochemical evidence for the etiology of SLD has not been revealed. Disruption of the balance between the oxidative status and the antioxidant status may lead to widespread oxidative damage in all tissues and organs and create many serious disease tables. Indeed, oxidative stress was accused of myelin deficiency and also learning dysfunction [28,29]. On the other hand there is not any research in the literature, that evaluates some oxidative stress parameters in children and their relationship with SLD. For this reason, we aimed to study the antioxidant and oxidant status, as well as other related laboratory parameters in children with SLD.

Despite all the searches we have done in the literature, no concrete research and findings have been reached on specific learning disorders regarding paraoxonase 1, arylesterase activities, native thiol and total thiol, ischemia modified albumin, total oxidant status, and total antioxidant status levels. For this reason, although there is a deficiency in the discussion part of our article, the original value of the article and its potential to be a source for new researches is very high. Displaying clinical laboratory parameters that may be associated with specific learning disorders may be very important for clarifying the etiology, investigating other related mechanisms, diagnosis, and treatment follow-up. Although there are no articles that can be similar to the measured parameters in our study, a limited number of studies including other parameters that may indicate oxidant and antioxidant status are discussed below.

In the light of the literature, we have seen that differences in metal levels may have both direct and oxidative stress-related effects and influence cognitive functions. It is known that maintaining a healthy diet and using vitamins are important for reducing the risk of cognitive impairment [9]. Although it has been claimed that people with learning difficulties have a higher risk of vitamin D deficiency in comparison to the general population, there are other publications that claim otherwise [10,11]. Vitamin B-12 is essential for brain development, as well as neural and cognitive function. It is known that inadequate vitamin B-12 levels in early childhood cause cognitive development disorders [12].

Looking at our findings, in the SLD group, the total oxidant status (p = .017), antioxidant enzymes PON1 (p = .045) and ARY (p < .001) levels significantly decreased relatively to the control. Additionally, when the thiol balance was evaluated, the SLD group was significantly different from the control, with lower values for reduced thiol ratio (p = .028), thiol oxidation reduction ratio (p = .01) and native thiols (p = .024) and with
higher values for oxidized thiol level \( (p = .028) \). The Oxidative Stress Index (OSI) value can be found by proportioning the serum Total Oxidant Level (TOS) value to the serum Total Antioxidant Level (TAS) value. The increase in antioxidant capacity in response to the increase in oxidative stress may mislead the measurement of TOS only in terms of causing oxidative damage, cell, tissue, and organ damage. By comparing the change in the TOS / TAS ratio, an accurate comparison is possible by eliminating the potentially misleading antioxidant reactive response for oxidative damage [21-23]. Thanks to TAS, TOS and OSI values, information and interpretation can be made about the oxidant-antioxidant capacity status, which is known to have a role in the etiology of many diseases, including pathologies with neurological and central nervous system involvement [21]. The antioxidant system with many components and the general evaluation of oxidative stress makes TAS, TOS measurement useful. For this reason, it is an important finding in our study that the OSI values are numerically higher in the SLD group and that this high OSI value is close to the statistically significant limit. In new clinical studies to be conducted by increasing the sample size, it is likely that a statistically significant value will occur in terms of OSI. Thiol contains a sulfhydryl group and is very effective in preventing oxidative stress. Reactive oxygen species oxidize thiol groups to form disulfide bonds. The dynamic thiol balance indicates the balance between thiol and disulfide. Maintaining this balance is very important in regulation of antioxidant capacity, detoxification, apoptosis, signal transduction and enzyme activities. Thiol oxidation produces reversible disulfide bonds, and if reduced, they may turn back into thiol bonds. Disruption of the balance between thiol and disulfide has been associated with the occurrence of many serious diseases. The thiol-disulfide balance may account for neurological disorders such as Parkinson’s, Alzheimer’s and Multiple Sclerosis [30,31]. No data related to the relationship between the thiol balance and SLD have been found in the literature. Based on our results, it was observed that such a balance was significantly impaired in oxidation in children with SLD. There is a possible relationship between zinc and copper levels and neurological disorders. For this reason, there is a possible relationship between zinc and copper levels and neurological disorders. In our measurements, the fact that vitamin D levels significantly declined for the SLD patient children differently Experimental and clinical studies. In literature, there is limited research and evidence on the likely association between SLD and oxidative stress, antioxidant status, antioxidant enzymes and thiol balance and 25 OH vitamin D. Our results are very significant in this respect.

CONCLUSION

In case of specific learning disorders, oxidative stress, antioxidant status and 25 OH vitamin D deficiency may be considered. The comparability of increasing oxidative stress and 25 OH vitamin D deficiency with SLD diagnosis in our results may be very important if it is supported by further experimental and clinical studies. In literature, there is limited research and evidence on the likely association between SLD and oxidative stress, antioxidant status, antioxidant enzymes and thiol balance and 25 OH vitamin D. Our results are very specific in this respect.

HIGHLIGHTS

According to the research results in this paper it was determined that:

- The deterioration resulting from oxidation in native-total thiol balance may be a laboratory indicator for pediatric specific learning disorders patients.
- Paraoxonase 1, arylesterase, which is the indicator of the antioxidant status are lower in pediatric specific learning disorders patients.
- Vitamin D deficiency may be considered as a laboratory indicator in pediatric specific learning disorders patients.
- The deterioration resulting from oxidation in total oxidant status may be a laboratory indicator for pediatric specific learning disorders patients.
Author contributions: All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

Funding: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Protection of humans: The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the 2013 Helsinki Declaration of the World Medical Association.

Declaration of interest: No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, shareholding and similar situations in any firm.

Data confidentiality: The authors declare having followed the protocols in use at their working center regarding patients' data publication.

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