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Evaluation of Thiol/Disulfide Homeostasis and Neurogenin 3 Levels as a Marker of Oxidative Stress in Children and Adolescents Newly Diagnosed with Type 1 Diabetes

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Abstract

The objective of the study was to examine thiol/disulfide homeostasis, neurogenin 3 (ngn 3) status in the newly diagnosed with type 1 diabetes mellitus (T1DM), and to investigate the changes in these parameters due to treatment. Totally 60 children aged 1-18 years old, 30 of them newly diagnosed with T1DM (patients), and 30 of them healthy children in the same age group (control) were included in the study. Insulin and C-peptide levels of newly diagnosed T1DM patients were lower than the control group, however HbA1c, glucose, creatinine, cholesterol, triglyceride and LDL levels were found higher in patients. Total thiol level and glutamic acid decarboxylase (GAD) antibody positive rate were found higher in children newly diagnosed with T1DM. In addition, it was observed that the rate of anti-insulin antibody positivity rate increased in the 6th month control of patients. However, it was determined that the HbA1c and glucose levels of the patient group decreased at the 6th month control. Insulin and C-peptide levels were found lower in patients admitted with diabetic ketoacidosis (DKA). A negative correlation was observed between native thiol and anti-insulin antibody parameters and a positive correlation between ngn 3 and total cholesterol, and LDL. In conclusion, thiol levels can be used as an oxidative stress marker in children newly diagnosed with T1DM and there was a significant difference between groups with or without DKA for insulin and C-peptide levels.

Keywords: Children, type 1 diabetes mellitus, thiol/disulfide, neurogenin 3, oxidative stress

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Introduction

Type 1 diabetes mellitus (T1DM) is a chronic metabolic disease most common in childhood and adolescents, characterized by insulin deficiency and hyperglycemia, resulting from damage to beta (β) cells of the pancreas due to autoimmune reasons. One of the mechanisms that are frequently emphasized in the etiopathogenesis of T1DM is oxidative stress.[1]

Highlights

- Abnormal thiol/disulfide balance is involved in the pathogenesis of diabetes mellitus.
- Few studies were available on the relationship between autoimmunity and dynamic thiol/disulfide, neurogenin 3 states in newly diagnosed with type 1 diabetes mellitus.
- Thiol/disulfide homeostasis and neurogenin 3 parameters can be used as oxidative stress markers in children and adolescents newly diagnosed with type 1 diabetes mellitus.

Thiols are organic compounds containing a sulfhydryl group consisting of a sulfur atom and a hydrogen atom attached to a carbon atom and have a critical role in preventing the formation of oxidative stress.[2] Thiols can be oxidized via oxidants and form disulfide bonds. The disulfide bonds formed can be reduced to thiol groups again, thus providing a dynamic thiol/disulfide homeostasis.[1] It has been reported that thiol/disulfide homeostasis has an important role in many physiological processes, such as detoxification, antioxidant defense, apoptosis, signal transduction, and stabilization of the chemical structures of proteins.[3] Abnormal thiol/disulfide homeostasis plays a role in the pathogenesis of many diseases such as diabetes, cancer, cardiovascular diseases, and rheumatoid arthritis.[1] Neurogenin 3 (ngn 3) is a pro-endocrine factor that plays a role in the determination of neural precursor cells in the neuroectoderm of the pancreas and is temporarily released by exocrine cells that are reprogrammed when the endocrine cell is damaged.[4]

The aim of this study was to determine the relationship between autoimmunity and dynamic thiol/disulfide, ngn 3 status in children with newly diagnosed T1DM, to examine thiol/disulfide homeostasis and ngn 3 level after insulin therapy and blood sugar regulation, and to evaluate the change in ngn 3 level due to beta-cell damage.

Material and Method

This study is a single-center, a prospective analytical study conducted with the permission of Aydın Adnan Menderes University Faculty of Medicine Ethics Committee, numbered 2019/21. Thirty patients aged 1-18 years who applied to Aydın Adnan Menderes University Faculty of Medicine Pediatric Endocrinology Clinic between 01 February 2019 and 01 February 2020 who newly diagnosed with T1DM and 30 healthy children of the same age group were included in the study. Detailed information was given to the subjects included in the study and their families, and a voluntary consent form was obtained. The study was conducted in accordance with the Declaration of Helsinki.

Serum insulin levels were measured by electrochemiluminescence immunoassay (ECLIA) technique (Abbott i2000). The C-peptide levels were measured on a Roche Modular Analytics Cobas 8000 Immunoassay analyzer with the ECLIA technique. The chromatographic/photometric method was used for HbA1c. Serum lipid profile, total cholesterol, HDL, and LDL levels were determined by enzyme colorimetric method, serum triglyceride level was measured by GPO/PAP (glycerine phosphate oxidase peroxidase) method using a commercial kit, and serum glucose level was determined by hexokinase method using a commercial kit (Abbott Architect c800). Anti-insulin antibodies were measured by the radioimmunoassay (RIA) method and glutamic acid decarboxylase (GAD) antibodies were measured by the immunoradiometric assay (IRMA) method. Plasma ngn 3 level and thiol/disulfide parameters were determined by ELISA (Enzyme-Linked Immunosorbent Assay) method using a commercial kit.

Anti-insulin and GAD antibody analysis of the patient group was repeated at the 6th month control to determine time-dependent changes.

Inclusion/exclusion criteria

Children aged 1-18 years old, newly diagnosed with type 1 diabetes (patients), and healthy children in the same age group (control) were included in the study. Patients with concomitant acute or chronic diseases, patients with a genetic syndrome, diabetes mellitus patients with the previous diagnosis, and children and adolescents under the age of 1 and above 18 years of age were excluded from the study.

Statistical analysis

SPSS (Statistical Package for Social Sciences) 22.0 program was used to analyze the data. Variables are presented as mean±standard deviation (SD), number (n), and percent (%). Kolmogorov-Smirnov test was applied to find the normality of the distribution of the numerical variables. For non-normally-distributed parameters Mann Whitney U-test or Kruskal Wallis test was performed, for normally distributed parameters Student t-test, or one-way ANOVA (analysis of variance). If there was a significant difference, the one-way ANOVA was applied to determine the arithmetic mean of a dependent variable between more than two independent groups. The relationship between a dependent variable and one or more independent variables was examined with logistic regression analysis.

Pearson's correlation coefficient has been preferred to determine whether there is a linear relationship between two numerical measurements when the data are normally distributed, and if so, in what direction and how strong that relationship is. If the data are not normally distributed, Spearman's correlation coefficient was preferred. Numerical variables with "mean±SD" and "median, 25-75% percentile" and categorical variables

with numbers and percentages were grouped together. The suitability of continuous variables for normal distribution was examined with visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). A Chi-square test was performed to show whether there was a difference between categorical variables in the study.

In independent groups, "Student t-test" for comparison of data with parametric properties and "Mann-Whitney U test" for comparison data without parametric properties were performed. In dependent groups, "T-Test for Dependent Groups" for comparison of data with parametric properties and "Wilcoxon Test" for the comparison of data without parametric properties were used. The P-values less than 0.05 were assumed significant.

Results

A total of 60 cases were included in the study. While 30 of them were newly diagnosed with T1DM, 30 were healthy (control). There was no statistical difference between the groups in terms of age and gender (p=0.975, p=0.999, respectively). Sixth-month examinations of 5 patients who did not come to their controls during the follow-up period could not be obtained. The characteristics and laboratory findings of the groups were shown in **Table 1**. The median insulin and C-peptide values of the newly diagnosed T1DM patients were lower than the control group (p<0.001). Furthermore, an increase was observed in HbA1c, glucose, creatinine, cholesterol, triglyceride, and LDL levels (p<0.001, 0.009, and 0.033, respectively) in the patient group. GAD antibody positivity was found to be higher in newly diagnosed with T1DM patients compared to the control group (p=0.001).

Plasma total thiol concentrations of newly diagnosed with T1DM and the control group were found 212.289±58.767 µmol/l and 186.058±32.862 µmol/l, respectively (p=0.038). Similarly, it was determined that native thiol levels tended to be higher in the patient group (p=0.052). However, no statistical difference was determined between the groups for other thiol/disulfide and ngn 3 parameters (**Table 2**).

Table 2.
The thiol/disulfide value of the groups

Item	Patient (n=30)	Control (n=30)	p
Total thiol (µmol/l) (mean±SD)	212.289±58.767	186.058±32.862	0.038
Native thiol (µmol/l) (mean±SD)	164.694±33.262	149.310±26.291	0.052
Disulfide (µmol/l) (mean±SD)	48.232±37.439	36.748±28.960	0.189
Disulfide/native thiol (%) (median) 25-75%	57.496 19.981-87.311	40.596 16.658-57.863	0.231
Disulfide/total thiol (mean±SD)	0.207±0.119	0.188±0.129	0.556
Native/total thiol (%) (mean±SD)	0.798±0.129	0.812±0.129	0.685
Neurogenin 3 (pg/ml) (median) 25-75%	32.431 (24.920-47.388)	33.896 (19.366-85.162)	0.941

Table 1.
The characteristics and laboratory findings of the groups

Item	Newly diagnosed with T1DM (n=30)	Control (n=30)	p
Age (year) (mean±SD)	9±4	9±4	0.975
Gender (male), n (%)	18 (60)	18 (60)	0.999
Height (cm) (mean±SD)	136±23	136±27	0.963
Height SDS (mean±SD)	-0.04±0.91	-0.26±1.07	0.385
Weight (kg) (mean±SD)	33±15	36±19	0.469
Weight SDS (mean±SD)	-0.34±1.13	-0.07±1.14	0.358
BMI (kg/m²) (mean±SD)	17.10±3.52	18.45±3.37	0.136
BMI SDS (mean±SD)	-0.50±1.43	0.08±1.01	0.075
Insulin (uU/ml) (median) 25-75%	2.15 1.40-2.70	7.65 4.10-11.90	<0.001
C-peptide (ng/ml) (median) 25-75%	0.22 0.16-0.34	1.39 0.82-1.80	<0.001
HbA1c (%) (median) 25-75%	12.5 11.5-13.2	5.2 5.1-5.5	<0.001
Glucose (mg/dl)(mean±SD)	422.17±169.34	83.33±12.29	<0.001
Urea (mg/dl)(mean±SD)	26±13	23±5	0.205
Creatinine (mg/dl) (median) 25-75%	0.84 0.76-1.03	0.61 0.52-0.65	<0.001
Total cholesterol (mg/dl) (mean±SD)	159±36	139±26	0.020
Triglyceride (mg/dl) (median) 25-75%	102 73-124	71 58-101	0.009
LDL (mg/dl) (median) 25-75%	94 69-117	76 61-97	0.033
HDL (mg/dl) (mean±SD)	40±12	46±10	0.074
Anti-insulin antibody, n			0.500
Negative	29 (96.7%)	30 (100.0%)	
Pozitive	1 (3.3%)	0 (0.0%)	
GAD antibody, n			0.001
Negative	15 (50.0%)	27 (90.0%)	
Pozitive	15 (50.0%)	3 (10.0%)	

GAD,glutamic acid decarboxylase

Laboratory findings of newly diagnosed with T1DM at diagnosis and at the sixth month control was shown in **Table 3**. HbA1c and glucose levels at diagnosis were found higher than at the sixth month control (p<0.001). There was no significant difference in thiol/disulfide parameters and ngn 3 between groups. It was determined that the anti-insulin antibody positivity of newly diagnosed T1DM patients increased at the 6th-month control compared to that at diagnosis (p=0.004).

It was determined that 63.3% of newly diagnosed with T1DM applied to clinics with diabetic ketoacidosis (DKA). There was a significant difference between groups with or without DKA for insulin and C-peptide levels (p=0.031 and p=0.011, respectively). For the thiol/disulfide parameters and ngn 3 levels no significant difference was determined between groups (**Table 4**).

The correlations of thiol/disulfide parameters with anthropometric data and laboratory tests are presented in **Table 5**. A negative correlation was found between native thiol and anti-insulin antibody parameters (r=-0.375, p<0.05), however, a positive correlation was found between ngn 3 and total cholesterol, and LDL (r=0.377, p<0.04; r=0.521, p<0.003, respectively).

Discussion

One of the most common chronic diseases of childhood is T1DM. In regions with a high incidence, it is more common in males.[5] Similarly, in the current study, it was determined that 60% of the newly diagnosed patients were male. In addition, similar to studies conducted in Turkiye,[6] the peak age of the disease was found to be

Table 4.
Laboratory findings of the patients who admitted with or without diabetic ketoacidosis (DKA)

Item	Group 1 without DKA (n=11)	Group 2 with DKA (n=19)	p
Insulin (uU/ml) (mean±SD)	4.19±3.09	1.83±0.71	0.031
C-peptide (ng/ml) (median) 25-75%	0.34 0.21-0.93	0.18 0.12-0.27	0.011
HbA1c (%) (mean±SD)	11.5±2.6	12.3±1.5	0.303
Glucose (mg/dl) (mean±SD)	392.82±236.85	439.16±119.18	0.555
Urea (mg/dl) (median) 25-75%	28 19-31	22 17-33	0.590
Creatinine (mg/dl) (mean±SD)	0.80±0.16	0.94±0.22	0.066
Total cholesterol (mg/dl) (mean±SD)	161±44	158±31	0.847
Triglyceride (mg/dl) (median) 25-75%	74 66-114	105 87-131	0.162
LDL (mg/dl) (median) 25-75%	97±37	97±28	0.983
HDL (mg/dl) (mean±SD)	44±13	39±10	0.271
Total thiol (µmol/l) (median) 25-75%	221.362 198.276-261.275	200.276 173.529-235.971	0.312
Native thiol (µmol/l) (mean±SD)	169.121±24.669	162.131±37.755	0.588
Disulfide (µmol/l) (median) 25-75%	47.787 41.075-56.196	22.586 10.614-72.330	0.292
Disulfide/native thiol (%) (median) 25-75%	61.882 51.090-82.333	25.966 11.561-98.113	0.292
Disulfide/total thiol (%) (median) 25-75%	0.216 0.195-0.250	0.130 0.077-0.305	0.333
Native/total thiol (%) (mean±SD)	0.767±0.058	0.816±0.154	0.232
Neurogenin 3 (pg/ml) (median) 25-75%	33.047 20.941-40.980	31.814 25.722-64.648	0.355

Table 3.
Laboratory findings of newly diagnosed with T1DM at admission and at the sixth month

Item	At diagnosis (n=25)	6th-month control (n=25)	p
C-peptide (ng/ml) (median) 25-75%	0.22 0.16-0.34	0.17 0.10-0.43	0.808
HbA1c (%) (median) 25-75%	12.5 11.6-13.6	7.0 6.4-7.6	<0.001
Glucose (mg/dl) (median) 25-75%	403 319-498	137 94-189	<0.001
Urea (mg/dl) (mean±SD)	25±14	27±7	0.257
Creatinine (mg/dl) (mean±SD)	0.89±0.23	0.62±0.08	0.307
Total cholesterol (mg/dl) (median) 25-75%	159 124-174	145 137-161	0.572
Triglyceride (mg/dl) (median) 25-75%	103 74-131	83 56-108	0.085
LDL (mg/dl) (median) 25-75%	94 69-117	72 66-87	0.067
HDL (mg/dl) (mean±SD)	39±12	54±12	0.076
Total thiol (µmol/l) (median) 25-75%	212.389 180.000-261.275	186.992 163.307-198.020	0.183
Native thiol (µmol/l) (mean±SD)	168.617±32.058	157.833±25.774	0.075
Disulfide (µmol/l) (mean±SD)	49.678±40.392	38.669±28.884	0.350
Disulfide/native thiol (%) (median) 25-75%	60.943 19.555-95.645	32.650 19.477-58.094	0.300
Disulfide/total thiol (%) (mean±SD)	0.204±0.126	0.197±0.126	0.994
Native/total thiol (%) (mean±SD)	0.802±0.136	0.865±0.193	0.371
Neurogenin 3 (pg/ml) (median) 25-75%	34.286 24.120-51.731	32.224 20.153-49.552	0.288
Anti-insulin antibody, n			0.004
Negative	24 (96.0%)	15 (60.0%)	
Pozitive	1 (4.0%)	10 (40.0%)	
GAD antibody, n			0.625
Negative	12 (48.0%)	10 (40.0%)	
Pozitive	13 (52.0%)	15 (60.0%)	

GAD,glutamic acid decarboxylase

Tablo 5.
The correlation of thiol/disulfide parameters with anthropometric data and laboratory tests in newly diagnosed with T1DM patients

Item	Total thiol		Native thiol		Disulfide		Disulfide/ native thiol		Disulfide/total thiol		Native/total thiol		Ngn 3	
	p	r	p	r	p	r	p	r	p	r	p	r	p	r
Age	0.734	0.065	0.345	-0.179	0.215	0.233	0.219	0.231	0.104	0.302	0.064	-0.342	0.074	-0.331
Height	0.845	0.037	0.332	-0.184	0.303	0.194	0.340	0.180	0.162	0.262	0.100	-0.306	0.142	-0.275
Height SDS	0.783	-0.052	0.905	0.023	0.602	-0.099	0.641	-0.089	0.594	-0.101	0.598	0.100	0.918	-0.020
Weight	0.931	0.016	0.423	-0.152	0.458	0.141	0.360	0.173	0.229	0.226	0.173	-0.256	0.259	-0.213
Weight SDS	0.866	0.032	0.480	0.134	0.775	-0.054	0.761	-0.058	0.836	-0.039	0.719	0.068	0.278	0.205
BMI	0.725	0.067	0.914	0.021	0.559	0.111	0.578	0.106	0.404	0.158	0.381	-0.166	0.657	0.085
BMI SDS	0.683	0.078	0.506	0.126	0.899	0.024	0.871	0.031	0.829	0.041	0.978	-0.005	0.090	0.315
Insulin	0.774	-0.055	0.148	-0.271	0.303	0.194	0.282	0.203	0.171	0.256	0.143	-0.274	0.200	-0.241
C-peptide	0.847	0.037	0.858	-0.034	0.599	0.100	0.551	0.113	0.466	0.138	0.381	-0.166	0.458	-0.141
HbA1c	0.592	0.102	0.892	0.026	0.504	0.127	0.426	0.151	0.557	0.112	0.505	-0.127	0.911	-0.021
Glucose	0.071	0.334	0.200	0.241	0.092	0.313	0.298	0.196	0.305	0.194	0.356	-0.175	0.235	0.223
Urea	0.061	0.346	0.079	0.326	0.195	0.243	0.177	0.253	0.361	0.173	0.328	-0.185	0.357	-0.174
Creatinine	0.546	0.115	0.757	-0.09	0.117	0.292	0.102	0.304	0.113	0.296	0.099	-0.307	0.646	0.087
Total cholesterol	0.497	0.129	0.454	0.142	0.691	0.076	0.232	0.225	0.576	0.106	0.592	-0.102	0.040	0.377
LDL	0.412	0.155	0.342	0.180	0.637	0.090	0.473	0.136	0.740	0.063	0.803	-0.047	0.003	0.521
Triglyceride	0.290	0.200	0.142	0.275	0.661	0.083	0.598	0.100	0.943	0.014	0.949	-0.012	0.392	0.162
HDL	0.098	-0.308	0.189	-0.247	0.134	-0.280	0.578	-0.106	0.518	-0.123	0.687	0.077	0.964	-0.009
Anti-insulin antibody	0.211	-0.235	0.041	-0.375	0.706	0.072	0.742	0.063	0.363	0.172	0.387	-0.164	0.134	0.280
GAD antibody	0.781	-0.053	0.226	-0.228	0.961	0.009	0.987	-0.003	0.939	0.015	0.882	-0.028	0.057	-0.351

BMI, body mass index, GAD,glutamic acid decarboxylase

10-14 years. In this study, the rate of admission with DKA to the clinic was 63.3%; HbA1c level was 12.5% (11.5-13.2), and glucose level was 422.17±169.34 mg/dl at admission. According to similar studies,[7,8] this increase in HbA1c might be related to the fact that the patients were diagnosed with DKA and longer insulinopenia.

Demiral et al. and Aras et al.[9,10] found the C-peptide level at diagnosis 0.57 and 0.82 ng/ml, respectively, and it was determined 0.22 ng/ml in the current study. Additionally, the C-peptide level of the group without DKA at admission was higher than the group with DKA. It can be thought that children with DKA at the time of admission have fewer residual beta cells and avoidance of DKA at admission is important for better residual beta cells.

In this study, thiol parameters (total and native) were found to be higher in children newly diagnosed with T1DM, and thiol/disulfide parameters were found to be statistically similar between the groups. Contrary to our findings, in previous studies, it was reported that thiol values were lower and disulfide/thiol ratios were higher in patients, and all of these studies were conducted in patients with has been diagnosed already.[11-13] In other words, it can be said while the patients in our study were exposed to acute stress, the patients in previous studies were exposed to chronic stress. It is known that humans and animals can adapt to chronic stress, and accordingly, their physiological responses and antioxidative defense mechanisms (such as thiol levels) can be changed against acute or chronic stress. However, the response of antioxidative defense can be affected by the severity and amount of stress too. The inconsistency between the data may be due to these physiological changes. Meanwhile, it can be thought that balance was in favor of thiol in the early stages of the disease and turned in favor of disulfide with time.

Although previous studies[11,12] showed that the increase in glucose and HbA1c changed the thiol/disulfide balance towards disulfide, no correlation was found between glucose and HbA1c and thiol/disulfide parameters in the current study. This may be according to the small number of patients in the study.

Çakıcı[14] stated that there is a positive correlation between disulfide parameters and total cholesterol. In the present study, no correlation was found between lipid parameters and thiol/disulfide parameters. Although it is known that hyperlipidemia increases oxidative stress, the lipid parameters of the patients in the current study were within the normal range.

In the present study, when the thiol/disulfide change was monitored prospectively, it was observed that there was a decrease in thiol/disulfide parameters over time but this was not statistically significant. This may be indicative of an acute inflammatory response due to the metabolic disturbance at the time of diagnosis. In addition, the decrease in native and total thiol levels compared to the admission may indicate that thiol/disulfide homeostasis shifted in favor of disulfide with the disease process. However, further studies with more patients are needed for definitive assumptions about the relationship between disease duration and oxidative stress.

Durmus et al.[11] reported that there was no relationship between the positivity of diabetes autoantibodies and thiol/disulfide parameters. But, a negative correlation was found between native thiol and anti-insulin antibody in the present study. According to these results, although it was thought that autoimmunity could increase oxidative stress, on the contrary, it was thought that oxidative stress could also increase autoimmunity. In order to distinguish between these two conditions, possible antioxidant treatment and evaluation of antibody titer may be required.

Ngn 3 is a pro-endocrine factor that is involved in the identification of neural precursor cells in the pancreatic neuroectoderm and is released by reprogrammed exocrine cells when the endocrine cell is damaged. Based on this, while the ngn 3 level was expected to be higher in patients, in the current study no statistical significant difference was determined between the patient and control groups. However, it was found that there was a positive correlation between ngn 3 and total cholesterol (p=0.040), and LDL (p=0.003), and also, total cholesterol (p=0.020) and LDL (p=0.033) levels were higher in patient group. This is the first study about ngn 3 levels in children newly diagnosed with T1DM and we believe that further studies are needed to clarified ngn 3 and other pathways in the differentiation stage from exocrine cell to endocrine cell.

Limitations of the study

The limitations of our study are the small sample size of the patient and control group, the dropout of five patients, the early counting of the sixth month in children newly diagnosed with T1DM for metabolic control, and the inability to compare thiol/disulfide homeostasis parameters with other oxidant and antioxidant markers.

Advantages of the study

This is the first study to evaluate the relationship between autoimmunity and thiol/disulfide homeostasis, ngn 3 status in newly diagnosed with T1DM and to investigate thiol/disulfide homeostasis and ngn 3 levels after insulin therapy and blood sugar regulation.

Conclusion

In conclusion, total thiol level and GAD antibody positive rate was found higher in children newly diagnosed with T1DM. The positive rate of the anti-insulin antibody of newly diagnosed T1DM patients increased at the 6th-month control compared to that at diagnosis. The HbA1c and glucose levels at diagnosis were found higher than at the sixth-month control in newly diagnosed T1DM patients. Insulin and C-peptide levels were determined lower in patients who were admitted with DKA. A negative correlation was found between native thiol and anti-insulin antibody parameters, however, a positive correlation was found between ngn 3 and total cholesterol, and LDL. According to these results, thiol levels can be used as oxidative stress markers in children newly diagnosed with T1DM, more studies should be conducted on thiol/disulfide homeostasis and ngn 3 levels, especially in these children.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Ethics Committee Approval: The study was carried out with the permission of Aydın Adnan Menderes University Faculty of Medicine Ethics Committee, numbered 2019/21.

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