The Large Effect Size of Urinary Total Antioxidant Capacity in Behavioral Symptoms of Young Autistic Individuals: Comparison with Omega-3 Fatty Acid and Superoxide Dismutase in Plasma

Kunio Yui1*, Hitomi Sasaki1, Nasoyuki Tanuma2 and Yohei Kawasaki3

1Department of Urology, School of Medicine, Fujita Health University, Toyoake, Japan
2Department of Pediatrics, Tokyo Metropolitan Fuchu Medical Center for the Disabled, Tokyo, Japan
3Chiba University Clinical Research Center, Chiba, Japan

Received date: Dec 21, 2017; Accepted date: Jan 17, 2018; Published date: Jan 27, 2018

Abstract

Objective: The imbalance between increased oxidative stress and reduced antioxidant defense has been implicated in the pathophysiology of autism spectrum disorders (ASD). Which of these has a greater impact on ASD behavioral symptoms is still unclear. We measured urinary levels of the oxidative stress biomarker hexanoyl-lysine (HEL), the total antioxidant capacity (TAC) and the DNA methylation biomarker 8-hydroxy-2′-deoxyguanosine (8-OHdG) and their relation to the plasma levels of the oxidative stress biomarker superoxide dismutase (SOD) and of the anti-inflammatory fatty acid eicosapentaenoic acid (EPA).

Methods: We studied the relationships between these biomarkers and behavioral symptoms in 19 individuals with ASD (mean age 10.9 ± 5.3 years) and 11 healthy controls (mean age 14.3 ± 6.3 years). Behavioral symptoms were evaluated using the Aberrant Behavior Checklist (ABC).

Results: Ages were not significant difference between two groups. The ASD group showed significantly reduced levels of urinary TAC and significantly increased levels of urinary HEL compared to the control group. Urinary 8-OHdG levels or plasma SOD and EPA levels were not significantly different between the two groups. The ABC subscale and total scores were significantly higher in the ASD group. There was significant correlation between the urinary TAC levels and plasma EPA levels and the ABC irritability scores.

Conclusion: Urinary TAP levels may be important in the imbalance between the urinary levels of HEL and TAC, and altered plasma SOD levels may contribute to this imbalance.

Keywords: Autistic disorders; Antioxidants; Oxidative stress; Superoxide dismutase; Eicosapentaenoic acid; Hexanoyl-lysine; 8-Hydroxy-2′-deoxyguanosine

Introduction

Accumulating evidence indicated that dyshomeostasis between antioxidant capacity and redox activity [1] and defects in detoxification systems [2] are implicated in the etiology of autism spectrum disorders (ASD). However, which factor has a greater impact on ASD behavioral symptoms remains unclear.

Previous studies reported findings on the set of oxidative stress markers in urine as follows: 1) elevated hexanoyl-lysine (HEL) levels in 24 children with ASD aged 5 to 12 years [3]; 2) higher 8-OHdG levels in 33 children with ASD aged 3–10 years [4]; 3) lowered urinary TAP levels in subjects with ASD in 29 subjects with ASD aged 6 to 12 years [5] or in 15 children with ASD aged 4 to 12 years [6]. Recently, a set consisting of the oxidative marker hexanoyl-lysine (HEL), total antioxidant capacity (TAC) and the DNA methylation marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine has been studied to examine the role of oxidative stress in brain damage [7-9]. There were few previous studies using a set of oxidative stress-related biomarkers such as HEL, TAC and 8-OHdG levels in urine.

A lot of former studies have reported that alterations in superoxide dismutase (SOD), an important main antioxidant enzyme [10], contribute to pathophysiology of ASD [11]. Erythrocyte SOD levels in ASD have been reported to decrease in serum [12] and plasma [13], and to increase in erythrocytes [10,11] and plasma [14]. Nevertheless, the relationship between plasma SOD levels and the urinary oxidative stress-related biomarkers is still unclear. In addition, ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) has anti-inflammatory capacity to resolve inflammation than DHA [15].

Taking these considerations together, we examined the links among HEL, TAC and 8-OHdG levels in urine, and their association to plasma levels of SOD and EPA in behavioral symptoms of juvenile individuals with ASD, and then estimated the effect sizes of these oxidative stress-related variables using standardized regression coefficients [16].
Urinary TAC levels are influenced by dietary intake [17]; therefore, dietary TAC was assessed in this study.

Methods

Subjects

Subjects were a total of 30 young, physically healthy individuals in Kobe and Osaka prefectures. The diagnosis of ASD was based on the DSM-5 criteria [18] and was confirmed by the Autism Diagnostic Interview-Revised (ADI-R) [19] by two physicians. Among the 30 participants, 19 were diagnosed with ASD (12 males and 7 females, mean age: 10.9 ± 5.3 years old, age range: 6-22 years old), and the rest of 11 were healthy normal controls (7 males and 4 females, mean age: 14.3 ± 6.4 years old, age range: 5-21 years old). These individuals with ASD showed the main symptoms of the DSM-5 diagnostic criteria for ASD, and had not any abnormal neurological symptoms (e.g., epileptic seizure or neuronal diseases). The 19 individuals with ASD and the 11 normal controls were matched on habits of dietary intake, age, gender and full intelligent quotient (IQ) scores (Table 1). These 30 subjects have no any extraordinary data in physical and laboratory examinations. The other criteria for inclusion were as follows: (a) no history of any other medical illnesses or comorbid psychiatric disorders; (b) a full IQ greater than 70 as estimated by the Wechsler Intelligence Scale Revised (WISC-R) [20] or the respective scale for adults [21] because high-functioning ASD were defined as subjects with an IQ above 70 required to have a total IQ of at least 70 [22]; and (c) no treatment with any other neuroleptics within the three months prior to the study.

This study was conducted according to the Ethics Committee approval of the Fujimoto Medical Clinic in Kobe City, Japan. As the most of the 30 subjects in the current study were youth under the legal age of 20 years; we gained parental permission and applied information on the behalf of these individuals. The participants and/or their parents provided written informed consent.

Sample size calculation

In this paper, Primary outcome was the Total Antioxidant Power (TAP). However, we provided estimated power of some parameters as below. Sample size calculation were calculated based on O'Brien-Castelloe approximation method for the Wilcoxon-Mann-Whitney test [23] using SAS 9.4 for Windows (SAS Institute Inc; Cary, NC).

TAP: Based on some previous study and studies using TAP, we estimated the mean difference of TAPs between the two groups as 1,000 points, with 850 (ASD group) and 150 (Control group) points of standard deviation (SD). Total sample size of urinary TAP levels was estimated as 30 for two groups, with a power level of 0.95 to detect a difference of TAPs and a 2-sided alpha level of 0.05.

HEL and 8-OHdG: We estimated the mean difference of HELs between the two groups as 25 points, with 30 (ASD group) and 25 (Control group) points of standard deviation (SD). Total sample size of urinary HEL was estimated as 30 for two groups, with a power level of 0.95 to detect a difference of HELs and a 2-sided alpha level of 0.05. Total sample size of urinary 8-OHdG levels was estimated as 30 for two groups, with a power level of 0.95 to detect a difference of 8-OHdG; and a 2-sided alpha level of 0.05.

SOD: We estimated the mean difference of SODs between the two groups as 1 points, with 3 (ASD group) and 3 (Control group) points of standard deviation (SD). Total sample size of plasma SOD levels was estimated as 30 for two groups, with a power level of 0.15 to detect a difference of SODs and a 2-sided alpha level of 0.05.

Urinary assay of HEL, 8-OHdG and TAC levels

Urine samples were collected as a spot sample and immediately stored at-80°C until analysis. After the dissolving process, the urines were centrifuged to remove all insoluble materials. The specialists at the Pediatrics, in Tokyo Metropolitan Fuchu Medical Center for the Disabled (Tokyo, Japan) assayed the urinary levels of HEL, 8-OHdG and TAC.

Urinary levels of HEL: The urinary HEL levels were measured in duplicate using a competitive ELISA kit (Japan International Cooperation Agency-JICA, Shizuoka, Japan) [24].

Urinary levels of 8-OHdG: Urine specimens were centrifuged and the supernatant after proper dilution was used in duplicate for assessment with a competitive enzyme-linked immunosorbent assay kit (8-OHdG check ELISA kit, JalCA). The urinary 8-OHdG/creatinine levels were used for analyses [25].

Urinary total antioxidant capacity: The urinary antioxidant capacity was determined by competitive enzyme-linked Immunosorbent assay (ELISA) [25].

Plasma levels of SOD: Plasma SOD levels were assayed using the reduce rate in nitrite induced by hydroxylamine and the superoxide anions based on the nitrite method (Molecular Devices Co, Tokyo, Japan).

Estimation of nutritional TAC: Urinary levels of TAC have been reported to be affected by dietary food intake [26]. Thus, a nutritional TAC value was assigned to each food item in the DHQ15.

Assessment of behavioral symptoms

The Aberrant Behavior Checklist (ABC) (Japanese version, Jiho, Ltd, Tokyo) was employed to evaluate the behavioral symptoms in the 19 individuals with ASD and the 11 normal controls. The ABC is a standardized rating scale for problematic behaviors for children and adolescents with normal IQ levels [27]. The ABC is a broad measure to assess a broad range of problematic behavior [28]. The internal consistency of the Japanese version based on Cronbach's alpha showed reliability ranged from .85 to .95 across five subscales [29] or from 0.75 to 0.98 [30], indicating high reliability of this version [28,29]. The Japanese version of the ABC showed good correlation with the Repetitive Behavior Scale-Revised (RBS-R) [31].

Moreover, irritability subscale scores of the Japanese version showed improvement similar to Clinical Global Impression in 47 ASD children who received aripiprazole compared to 45 children who received placebo [32]. With respect to validity of the ABC, a previous review article reported that a few of the seven behavioral showed test-retest and inter-rater reliability and structural validity scales for assessing behavioral symptoms in ASD [33], however, one recent study described the cross-cultural stability and validation for assessment of overall behavior symptoms in the ABC [34]. Additionally, the Brazilian Portuguese version of the ABC showed cross-cultural stability of the ABC [35].
Statistical analyses

As the data were not normally distributed, the non-parametric Mann-Whitney U test for multiple comparisons was employed to elucidate significant differences between the groups. Multiple regression analysis was conducted to determine the relationship between the urinary (HEL, 8-OHdG and TAC) and plasma oxidative stress-related biomarkers (SOD and EPA), and the other variables (two groups, and the ABC scores) (Table 2).

Importantly, to estimate the effects size of the urinary and plasma oxidative stress-related biomarkers, standardized regression coefficients were employed. The standardized coefficients were useful to determine the most important predictor variables [16]. We conducted statistics using SPSS version 18.0 (IBM Tokyo, 2009).

Results

Study population

Behavioral symptoms of the 19 individuals with ASD were characterized by repetitive patterns of interest and activities (n=8), social withdrawal (n=8) and irritability (n=3). The mean ABC score was 62.7 ± 30.0 (Table 1).

According to a previous study, total ABC scores were 60.1 in children and adolescents with moderate or severe ASD [36], and 83.4 ± 31.8 in 18 children with ASD who had severe maladaptive behaviors such as self-injurious behavior [37]. Thus, the patients in this study suffered moderate or severe behavioral symptoms. Ages were not significantly different between the two groups.

Oxidative stress marker levels in urine

The Mann-Whitney U-test revealed that a significant increase in urinary HEL levels and a significant decrease in urinary TAC levels in the 19 individuals with ASD as compared to the 11 normal controls. There were no significantly differences in urinary 8-OHdG levels and plasma SOD and EPA levels between the groups (Table 1).

| Variable                        | ASD (n=19)      | Control (n=11) | U    | P-value |
|---------------------------------|----------------|---------------|------|---------|
| Age (years)                     | 10.9 ± 5.3     | 14.3 ± 6.4    | 3    | 0.19    |
| Sex (male/female)               | 13-Jun         | 4-Jul         | χ²=0.00 | 1.00    |
| Full IQ                         | 100.9 ± 31.4   | 112.6 ± 17.4  | 27.5 | 0.33    |
| Scores of ADI-R                 |                |               |      |         |
| Domain A (social)               | 13.3 ± 6.3     |               |      |         |
| Domain B (communication)        | 7.4 ± 5.3      |               |      |         |
| Domain C (stereotyped behavior) | 11.2 ± 6.0     |               |      |         |
| Urinary levels                  |                |               |      |         |
| HEL                             | 75.55 ± 31.00  | 51.18 ± 26.09 | 55   | 0.033*  |
| 8-OHdG                          | 11.27 ± 5.64   | 9.62 ± 3.35   | 2.5  | 0.61    |
| TPA                             | 2969.87 ± 820.14 | 4152.85 ± 131.60 | 56   | 0.037*  |
| Plasma SOD                      | 3.87 ± 3.26    | 3.58 ± 2.65   | 87.5 | 0.47    |
| Subscores of ABC                |                |               |      |         |
| Irritability                    | 13.37 ± 8.00   | 0.73 ± 1.10   | 1    | 0.000** |
| Social withdrawal               | 19.32 ± 10.13  | 0.36 ± 0.92   | 2    | 0.000** |
| Stereotypy                      | 4.53 ± 4.42    | 0.36 ± 0.67   | 23   | 0.000** |
| Hyperactivity                   | 20.37 ± 11.70  | 0.91 ± 2.12   | 3    | 0.000** |
| Inappropriate speech            | 4.68 ± 3.38    | 0.27 ± 0.65   | 17.5 | 0.000** |
| Total                           | 62.79 ± 29.93  | 2.45 ± 4.85   | 0.5  | 0.000** |

*p<0.05 and **p<0.001, significant differences

Table 1: Subject characteristics; urinary HEL, 8-OHdG and TAC levels; and the ABC scores in the 19 individuals with ASD and the 11 normal controls. ADI-R, Autism Diagnostic Interview-Revised; ABC, Aberrant Behavior Checklist; HEL, hexanolyl-lysine; 8-OHdG, 8-hydroxy-2’-deoxyguanosine; TPA, total antioxidant power; and SOD; superoxide dismutase.
Magnitudes of variables

As shown in Table 2, stepwise regression analysis indicated a significant association of urinary TAP with two groups. Meanwhile, urinary HEL levels and plasma SOD and EPA levels were significantly associated with the ABC irritability scores. The group, as used as a dependent variable, made a significant contribution to the urinary TAP levels. The ABC irritability scores as used dependent variables were significantly associated with urinary HEL levels, plasma SOD and EPA levels. Importantly, urinary TAP levels contributed to discriminating the ASD group from the control group.

The standardized regression coefficients indicated that effect size of each oxidative stress related variables in order of large effect size as TAP, HEL, SOD, EPA, and 8-OHdG. Thus, the urinary TAC levels have larger effect size that is more powerful compared to urinary HEL (Table 2).

Dietary total antioxidant power

The ASD group absorbed significantly more dietary TAC in the form of chocolate (p=0.02), cookies and biscuits (p=0.04), and jam and marmalade (p=0.007) than in the control group. While, there were no significant differences between dietary TAC levels and TAP urinary levels (r=0.02–0.57, p=0.95–0.053) in the ASD group.

Discussion

Urinary TAC levels were significantly reduced, whereas urinary HEL levels were significantly increased, in the ASD group compared with the control group. Importantly, the standardized regression coefficients indicated that urinary TAC levels showed a larger effect size compared with urinary HEL levels or plasma SOD and EPA levels.

Thus, urinary TAC levels were a more powerful explanatory variable than the urinary HEL levels, indicating first order multiple linear regression models for distinguishing the two groups.

Table 2: Results from the stepwise regression analysis. TAP=total antioxidant power; R²=R-squared values; B=unstandardized coefficients; ASD=autism spectrum disorder; ABC=Aberrant Behavior Checklist and EPA=Eicosa-pentaenoic acid.

| Model                        | R²   | P value | Coefficients B | Beta  | P-value |
|------------------------------|------|---------|----------------|-------|---------|
| TAP                          | 0.283| 0.002*  | 1282.98 ± 385.900 | 0.532 | 0.002*  |
| Group (1=ASD; 2=control)     |      |         |                |       |         |
| Standardized regression Coefficient | -0.8578 | 0.011* |                |       |         |
| HEL                          | 0.222| 0.099*  |                |       |         |
| ABC irritability score       |      |         | 1.661 ± 0.587  | 0.46  | 0.009*  |
| Standardized regression Coefficient | 0.2702 | 0.4347 |                |       |         |
| SOD                          | 0.4741| 0.000** |                |       |         |
| ABC irritability score       |      |         | 0.185 ± 0.067  | -0.553| 0.010*  |
| Standardized regression Coefficient | -0.1354 | 0.6289 |                |       |         |
| EPA                          | 0.433| 0.017*  |                |       |         |
| ABC irritability score       |      |         | 0.997 ± 0.392  | 0.443 | 0.017*  |
| Standardized Regression Coefficient | -0.1065 | 0.7631 |                |       |         |

*p<0.05 and **p<0.001, significant contribution

Previous studies reported findings on the set of oxidative stress markers in urine as follows: 1) elevated urinary hexanoyl-lysine (HEL) levels in 24 children with ASD aged 5 to 12 years, while other oxidative stress marker such as urinary8-hydroxy-2′-deoxyguanosine (8-OHdG) levels or erythrocytes SOD levels were unchanged [3] suggesting oxidative stress and erythrocyte membrane alterations as a role of the pathogenesis of ASD [3]; 2) significantly higher levels of urinary 8-OHdG levels in 40 children with ASD aged 3–10 years as compared with 40 age-matched normal controls [4]; 3) lowered activity of urinary total antioxidant capacity (TAP) and total thiol molecules which has antioxidant capacity [38] concomitant with higher urinary catalase activity, which is regulated by oxidative stress [39], in 29 subjects with ASD aged 6 to 12 years compared with 24 normal controls [5]. This study suggested that increased vulnerability to oxidative stress may contribute to the development and clinical symptoms of ASD [5]. Additionally, urinary TAC levels were significantly lower in 45 ASD children aged 4-12 years as compared with age matched controls [6]. Collectively, it is unclear which of these markers has a greater impact on ASD symptoms. The present study firstly revealed that urinary TAC levels were a more powerful explanatory variable than the urinary HEL levels.

The significant increase in urinary HEL levels and the significant reduction in urinary TAC levels in the 19 individuals with ASD indicated an imbalance between oxidative stress and antioxidant capacity. Moreover, stepwise regression analysis revealed that the urinary level of TAC was a reliable index for distinguishing the two groups. A former review article indicated that the imbalance between reactive oxygen species (ROS) production in relation to oxidative stress and TAC may correlate with pathophysiology of ASD [1,40]. However, the important question of whether oxidant or antioxidant factors have a stronger impact remained unclear. The present findings firstly reveal that urinary TAP levels had a greater impact than the urinary levels of HEL and plasma levels of SOD and EPA on the imbalance between TAP (antioxidant) and HEL (oxidant) in urine.
Our stepwise multiple regression analysis indicated that plasma SOD levels contributed significantly to ABC irritability. However, further studies will be needed to elucidate the effect of plasma SOD levels on ABC subscale scores. Stepwise regression analysis indicated significant correlation of plasma omega-3 fatty acid EPA levels and ABC irritability scores. Several clinical studies reported that low dose omega-3 fatty acids including EPA decreased irritability in 19 subjects with bipolar depression, indicating therapeutic effects on irritability of psychiatric condition [41], and that closely relationship between low plasma levels of omega-3 fatty acids and vulnerability for irritability [42]. These previous studies may support our above findings (Figure 1).

Figure 1: The findings and limitations in this study.

Oxidative stress was concerned with various pathophysiological conditions, and the human body prevent gainst the harmful effects of oxidative stress damage with the the antioxidant defense system which comprises both enzymatic and nonenzymatic mechanisms [43,44]. Such systems include the endogenous antioxidant system (43,44). Most previous studies on antioxidant capacity in individuals with ASD have suggested deficient antioxidant defense mechanism especially at young children [45] or a chronically low detoxifying capacity [46]. These antioxidant defense systems may be a part of the endogenous antioxidant systems. Indeed, recent research on antioxidant networks has demonstrated that antioxidant enzymes such as SOD, glutathione peroxidase, and glutathione act as an antioxidant network within specific intracellular or extracellular components of the antioxidant system [47]. Further, a recent report has suggested that the autophagolysosomal activities of these antioxidant enzymes may serve an essential function in preventing neurodegenerative diseases by removing damage as part of an essential cellular antioxidant pathway [48]. Taking these considerations together, the endogenous antioxidant system may be deficient in individuals with ASD. While a former clinical study on TAC in plasma indicated that in a group of adolescents with Asperger syndrome, impaired detoxifying capacity was not found in the first years of illness despite of chronic low detoxifying capacity [46]. Impaired detoxifying capacity may therefore c oxidative stress damage. Further studies will be need to confirm which specific factor may be impaired and whether deficit TAC is intrinsic impairment or secondary deficient resulting from oxidative stress in ASD.

The ASD group revealed significantly higher dietary TAC for chocolate, biscuits and cookies and jam and marmalade as compared with the control group. Cocoa products including chocolate [49], cookies containing chocolate chips [50], and jam and marmalade [51] increase antioxidant capacity. Reduced urinary TAC levels in the ASD group suggest that antioxidant defense systems i.e., endogenous antioxidant defense system [44] or intrinsic antioxidant defenses [47] may be deficit.

Excess oxidative stress against the endogenous antioxidant defense sometimes induced disruption of the blood-brain barrier (BBB), and then brain-specific proteins circulating inside the brain are observed in the peripheral blood as an index for the increase in BBB permeability and brain damage [52]. Overproduction of ROS and the imbalance between ROS and antioxidant capacity induces toxic effects on brain neurons. Thus, imbalance between increased HEL levels and decreased TAC levels in urine may interrupt the BBB, resulting behavioral abnormalities in the ASD group.

This study has some limitations. Firstly, previous studies examined urinary oxidative stress biomarkers such as F2-isoprostanes and their association with the activity of plasma enzymatic antioxidants such as SOD and glutathione peroxidase [53]. In current study, an informative set of oxidative stress-related biomarkers were examined, and this work revealed novel, important information on impaired antioxidant capacity that was not reported in previous studies [3,5,13]. Secondly, ASD is most prevalent in males, with a male to female ratio of 4 to 1 [53]; however, in the current study, the ASD and control groups were matched for age and gender, suggesting influence of on the male to female ratio. Finally, the small sample size limit the ability to generalize findings our findings to all patients with ASD.

Conclusion

This study firstly report that reduced levels of TAC and increased levels of HEL in urine may be conductive to the behavioral sequence in individuals with ASD, without significant changes in urinary 8-OHdG levels. Importantly, reduced urinary TAC levels could be preferentially used for distinguishing the ASD group from the control group. The endogenous antioxidant systems may be impaired in young subjects with ASD.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (Grant No 21200017) (2010-2012) and a Grant-in-Aid for Scientific Research (C) (2014-2016) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, et al. (2006) Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsyiatr Genet 141B: 947-956.
2. Alabdali A, Al-Ayadhi L, El-Ansary A (2014) A key role for an impaired detoxification mechanism in the etiology and severity of autism spectrum disorders. Behav Brain Funct 28: 10: 14.
3. Ghezzo A, Visconti P, Abruzzo PM, Bolotta A, FERRERI C, et al. (2013) Oxidative Stress and erythrocyte membrane alterations in children with autism: correlation with clinical features. PLoS One 8: e66418.
4. Melnyk S, Fuchs GI, Schulz E, Lopez M, Kahler SG, et al. (2012) Metabolic imbalance associated with methylation dysregulation and
oxidative damage in children with autism. J Autism Dev Disord 42: 367-377.
5. Ranjarb A, Rashdi V, Rezaei M (2014) Comparison of urinary oxidative biomarkers in Iranian children with autism. Res Dev Disabil 5: 2751-2755.
6. Dammadoran LP, Arumugam G (2011) Urinary oxidative stress markers in children with autism. Redox Rep 16: 216-222.
7. Tokuda F, Sando Y, Matsui H, Yokoyama TN (2009) N epsilon-(hexanoyl) lysine, a new oxidative stress marker, is increased in metabolic syndrome, but not in obstructive sleep apnea. Am J Med Sci 338: 127-133.
8. Hamada A, Esteves SC, Aragaw A (2013) Insight into oxidative stress in varicocele-associated male infertility: part 2. Nat Rev Urol 10: 26-37.
9. Miyata R, Tanuma N, Sakuma H, Hayashi H (2016) Circadian Rhythms of Oxidative Stress Markers and Metabolism Medikote in Patients with Xeroderma Pigmentosum Group A. Oxid Med Cell Longev 5741517.
10. Hung TH, Lo LM, Chiu TH, Li MJ, Yeh YL, et al. (2010) A longitudinal study of oxidative stress and antioxidant status in women with uncomplicated pregnancies throughout gestation. Reprod Sci 7: 401-409.
11. Zoroglu SS, Armutcu F, Ozen S, Gurel A, Sivasli EO, et al. (2004) Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism. Eur Arch Psychiatry Clin Neurosci 254: 143-147.
12. Yorbik O, Sayal A, Akay C, Akbıyık DI, Sohmen T (2002) Investigation of antioxidant enzymes in children with autistic disorder. Prostaglandins Leukot Essent Fatty Acids 67: 341-343.
13. Kondolot M, Ozmert EN, Ascı A, Erkekoglu P, Oztop DB, et al. (2016) Plasma phthalate and bisphenol a levels and oxidant-antioxidant status in autistic children. Environ Toxicol Pharmacol 43: 149-158.
14. Morin C, Blier PU, Fortin S (2015) Eicosapentaenoic acid and docosapentaenoic acid monoglycerylces are more potent than docosahexaenoic acid monoglyceride to resolve inflammation in a rheumatoid arthritis model. Arthritis Ther Res 17: 142.
15. Kim RS (2011) Standardised regression coefficients as indices of effect sizes in meta-analysis. Florida State University Libraries, The Florida State University College of Education, Florida.
16. Scholl TO, Leskiv M, Chen X, Sims M, Stein TP (2005) Oxidative stress, diet, and the etiology of preeclampsia. Am J Clin Nutr 81: 1390-1396.
17. American Psychiatric Association (2015) Diagnostic and statistical manual of mental disorders. 5th edn. American Psychiatric association, Washington, DC.
18. Rutter M, Le Couteur A, Lord C (2003) ADI-R Autism Diagnostic Interview Revised. Manual. Western Psychological Services, Los Angeles.
19. Wechsler D (1974) Wechsler Intelligence Scale for Children-Revised Manual. The Psychological Corporation, New York, NY.
20. Wechsler D (1981) Wechsler Intelligence Scale for Children-Revised Manual of mental disorders. 5th edn. American Psychiatric association, Florida State University College of Education, Florida.
21. Koyama T, Kamio Y, Inada N, Kurita H (2009) Sex differences in WISC-III profiles of children with high-functioning pervasive developmental disorders. J Autism Dev Disord 39: 135-141.
22. Tang Y (2011) Size and power estimation for the Wilcoxon-Mann-Whitney test for ordered categorical data. Stat Med 30: 3461-3470.
23. Matsui Y, Satoh K, Miyazaki T, Shirabe S, Atarashi R, et al. (2011) High sensitivity of an ELISA kit for detection of the gamma-isoform of 14-3-3 proteins: usefulness in laboratory diagnosis of human prion disease. BMC Neurol 11: 120.
24. Boonla C, Wünsuwan R, Tungsanga K, Tosukhowong P (2007) Urinary 8-hydroxydeoxyguanosine is elevated in patients with nephrolithiasis. Urol Res 35: 185-191.
25. Kobayashi S, Murakami k, Sasaki S, Uenish K, Yamasaki M, et al. (2011) Dietary total antioxidant capacity from different assays in relation to serum C-reactive protein among young Japanese women. Nut J 11: 91.
26. Hollander H, Chaplin W, Soorya L, Wasserman S, Novotny S, et al. (2009) Divalproex sodium vs. placebo for the treatment of irritability in children and adolescents with autism spectrum disorders. Neuropsychopharmacology 35: 990-998.
27. Karabekiroglu K, Aman MG (2009) Validity of the aberrant behavior checklist in a clinical sample of toddlers. Child Psychiatry Hum Dev 40: 99-110.
28. Ono Y (1996) Factor validity and reliability for the Aberrant Behavior Checklist-Community in a Japanese population with mental retardation. Res Dev Disabil 17: 303-309.
29. Uesugi M, Naruse S, Yuri Inoue Y, Koeda H, Gotou M, et al. (2012) Examining the Intra-rater reliability of the Japanese version of the Aberrant Behavior Checklist used for handicapped children. J Phys Ther Sci 24: 1115-1117.
30. Inada N, Ito H, Yasunaga K, Kuroda M, Iwanaga R, et al. (2015) Psychometric properties of the Repetitive Behavior Scale-Revised for individuals with autism spectrum disorder in Japan. Res Autism Spectr Disord 15-16: 60-69.
31. Ichikawa H, Mikami K, Okada T, Yamashita Y, Ishizaki Y, et al. (2017) Aripiprazole in the Treatment of Irritability in Children and Adolescents with Autism Spectrum Disorder in Japan: A Randomized, Double-blind, Placebo-controlled Study. Child Psychiatry Hum Dev 48: 796-806.
32. Hanratty J, Livingston N, Robalino S, Terwee CB, Glod M, et al. (2015) Systematic Review of the Measurement Properties of Tools Used to Measure Behaviour Problems in Young Children with Autism. PLoS One 10: e0144649.
33. Kaat AJ, Lecavalier L, Aman MG (2014) Validity of the aberrant behavior checklist in children with autism spectrum disorder. J Autism Dev Disord 44: 1103-1116.
34. Losapio MF, Silva LG, Póndé MP, Novac CM, dos Santos DN, et al. (2011) Partial cross-cultural adaptation of the Aberrant Behavior Checklist (ABC) scale for analysis of patients with mental retardation. Cad Saúde Pública 27: 909-923.
35. Singh K, Connors SL, Macklin EA, Smith KD, Fahey JW, et al. (2014) Sulforaphane treatment of autism spectrum disorder (ASD). Proc Natl Acad Sci USA 111: 15350-15355.
36. Weiner RH, Greene RL (2014) Intention-based therapy for autism spectrum disorder: promising results of a wait-list control study in children. Explore (NY) 10: 13-23.
37. Balcerekza Y, Bartosz G (2003) Thiols are main determinants of total antioxidant capacity of cellular homogenates. Free Radic Res 37: 537-541.
38. Cao C, Leng Y, Kufe D (2003) Catalase activity is regulated by c-Abl and Arg in the oxidative stress response. J Biol Chem 278: 15550-15555.
39. Papadiana S, Soriano FX, Léveillé F, Martel MA, Dakin KA, et al. (2008) The Large Effect Size of Urinary Total Antioxidant Capacity in Behavioral Symptoms of Young Autistic Individuals: Comparion with Omega-3 Fatty Acid and Superoxide Dismutase in Plasma. J Child Adolesc Behav 6: 367.
40. Meguid N, Dardir AA, Abdel-Raouf ER, Hashish A (2011) Evaluation of dietary total antioxidant capacity from omega-3 fatty acids. J Physiosc Res 75: 475-483.
41. Bhattacharyya A, Chattopadhay R, Mitra S, Crowe SE (2014) Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 94: 329-354.
42. Miglio C, Pelis I, Raguzzini A, Villano DV, Cesqui E, et al. (2005) Omega-3 fatty acids decreased irritability of patients with bipolar disorder in an add-on, open label study. Nutr J 4: 6.
47. Liu S, Li X, Wu S, He J, Pang C, et al. (2014) Fungal pretreatment by phanerochaete chrysosporium for enhancement of biogas production from corn stover silage. Appl Biochem Biotechnol 174: 1907-1918.

48. Todorovic V, Redovnikovic IR, Todorovic Z, Jankovic G, Dodevska M, et al. (2015) Polyphenols, methylxanthines, and antioxidant capacity of chocolates produced in Serbia. J Food Composit Anal 41: 137-143.

49. Sun Y, Hayakawa S, Ogawa M, Fukada, Izumori K (2008) Influence of a rare sugar, d-Psicose, on the physicochemical and functional properties of an aerated food system containing egg albumen. J Agric Food Chem 56: 4789-4796.

50. Kamiloglu S, Pasli AA, Ozcelik B, Van Camp J, Capanoglu E (2015) Influence of different processing and storage conditions on in vitro bioaccessibility of polyphenols in black carrot jams and marmalades. Food Chem 186: 74-82.

51. Hee-Tae H, Su-Youn C, Wi-Young S (2016) Obesity promotes oxidative stress and exacerbates blood-brain barrier disruption after high-intensity exercise. J Sport Health Sci 1-6.

52. Matayatsuk C, Lee CY, Kalpravidh RW, Sirankapracha P, Wilaira P, et al. (2007) Elevated F2-isoprostanes in thalassemic patients. Free Radic Biol Med 43: 1649-1655.

53. Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, et al. (2012) Why are autism spectrum conditions more prevalent in males? PLoS Biol 9: e1001081.