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Plasma phthalate and bisphenol a levels and oxidant-antioxidant status in autistic children

Meda Kondolot[a,*], Elif N. Ozmert[b], Ali Ascı[c], Pınar Erkekoglu[c], Didem B. Oztop[d], Hakan Gumus[e], Belma Kocer-Gumusel[c,**], Kadriye Yurdakok[b]

[a] Erciyes University Faculty of Medicine, Department of Pediatrics, Social Pediatrics Unit, Kayseri 38039, Turkey
[b] Hacettepe University Faculty of Medicine, Department of Pediatrics, Social Pediatrics Unit, Developmental Pediatrics Unit, Ankara 06100, Turkey
[c] Hacettepe University Faculty of Pharmacy, Department of Toxicology, Ankara 06100, Turkey
[d] Erciyes University Faculty of Medicine, Department of Child Psychiatry, Kayseri 38039, Turkey
[e] Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Kayseri 38039, Turkey

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ABSTRACT

Phthalates and bisphenol A (BPA) are endocrine disruting chemicals (EDCs) that are suggested to exert neurotoxic effects. This study aimed to determine plasma phthalates and BPA levels along with oxidant/antioxidant status in autistic children [n = 51; including 12 children were diagnosed with "Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS)]. Plasma levels of BPA, di (2-ethylhexyl)-phthalate (DEHP) and its main metabolite mono (2-ethylhexyl)-phthalate (MEHP); thiobarbituric acid reactive substance (TBARS) and carbonyl groups; erythrocyte glutathione peroxidase (GPx1), thioredoxin reductase (TrxR), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) activities and glutathione (GSH) and selenium levels were measured. Plasma BPA levels of children with PDD-NOS were significantly higher than both classic autistic children and controls (n = 50). Carbonyl, selenium concentrations and GPx1, SOD and GR activities were higher ($p < 0.05$); CAT activity was markedly lower in study group. BPA exposure might be associated with PDD-NOS. Intracellular imbalance between oxidant and antioxidant status might facilitate its neurotoxicity.

1. Introduction

Prevalence of 'Autism Spectrum Disorders (ASDs)' has increased dramatically (1 in 68 in United States) (Centers for Disease Control and Prevention, 2011). However, their etiology is not well understood and it is hypothesized that both genetic and environmental factors are involved. Chemicals such as lead, methylmercury, organic solvents, endocrine disrupting chemicals (EDCs), and pesticides are suspected causes of developmental neurotoxicity (Grandjean and Landrigan, 2006), though there are no well-designed studies about the role of these chemicals in ASDs.

Endocrine disrupting chemicals are man-made chemicals that disrupt the physiological function of endogenous hormones (World Health Organization, 2012). Phthalates and bisphenol A (BPA) are the most abundant EDCs in the environment. These substances are plasticizers that are available in a large number of consumer products. Humans are exposed to these chemicals mainly by food and drink. On the other hand, dermal and indoor air exposure are also important sources (World Health Organization, 2012; Braun et al., 2013; Rubin, 2011). Phthalates are anti-androgenic chemicals that are present in many commercial products such as personal-care products, plastic materials, food packages, detergents and paints. The most abundant phthalate in food and environment is di-(2-ethylhexyl)-phthalate (DEHP) and its major metabolite is mono-(2-ethylhexyl)-phthalate (MEHP) (World Health Organization, 2012; Braun et al., 2013). BPA is a plasticizer that is used to harden plastics. It is regarded as a weak

Abbreviations: ASDs, autism spectrum disorders; BPA, bisphenol A; CAT, catalase; CV, coefficient of variation; DEHP, di-2-ethylhexyl-phthalate; DSM-V, diagnostic and statistical manual of mental disorders-V; EDCs, endocrine disrupting chemicals; GPx1, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; HPLC, high performance liquid chromatography; H_2O_2, hydrogen peroxide; LOD, limit of detection; LOQ, limit of quantification; LP, lipid peroxidation; MDA, malondialdehyde; MEHP, mono-2-ethylhexyl-phthalate; PCV, polyvinyl chloride; PDD-NOS, pervasive developmental disorder-not otherwise specified; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; TNB, 5-thio-2-nitrobenzoic acid; TrxR, thioredoxin reductase; WST-1, cell proliferation reagent; XO, xanthine oxidase.

* Corresponding author.
** Corresponding author.
E-mail addresses: medakondolot@gmail.com (M. Kondolot), belmagumusel@yahoo.com (B. Kocer-Gumusel).

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estrogenic chemical that is available in a large number of consumer products (World Health Organization, 2012; Rubin, 2011).

Neurotoxic mechanisms of EDCs or timing of this particular toxic effect are not clearified yet. As phthalates have both anti-androgenic or weak estrogenic activity and BPA exerts weak estrogenic activity, they might interfere with hormone-sensitive periods of neural development such as neuronal differentiation, growth, and synapse formation (Fox et al., 2010; Tareen and Kamboj, 2012; Colborn, 2004). Recent studies reported that phthalates were associated with decreased mental and psychomotor index scores (Kim et al., 2011; Whyatt et al., 2012; Téllez-Rojo et al., 2013), behavior and emotional problems (Whyatt et al., 2012; Engel et al., 2010; Swan et al., 2010), social impairment (Miodovnik et al., 2011), *attention deficit hyperactivity disorder* (Kim et al., 2009). On the other hand, BPA was associated with behavioral problems (Fox et al., 2010; Braun et al., 2009; Braun et al., 2011; Perera et al., 2012). Moreover, two separate studies, which reported higher urinary concentrations of some DEHP metabolites and BPA in autistic children compared with healthy controls, were published before (Stein et al., 2015; Testa et al., 2012). Only few studies showed that these EDCs induced oxidative stress and imbalance in oxidant/antioxidant status (Kaur et al., 2014; Erkekoglu et al., 2014).

Taking into account all the available data, we aimed to evaluate plasma phthalates and BPA levels and the oxidant/antioxidant status in autistic children. To our knowledge, this is the first study investigating DEHP, MEHP and BPA exposure in autistic children along with oxidant/antioxidant status.

2. Materials and methods

2.1. Subjects

The study group comprised of 51 autistic children (mean age: 5.8 ± 2.5 years), admitted to Erciyes University Child Psychiatry Clinic in Kayseri between July 2011 and August 2012. Autistic children with associated neurologic, metabolic and genetic disorders were excluded from the study group. Since Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) had not been published yet, all the patients with ASDs were diagnosed according to DSM-IV-Text Revision (APA, 2013) and Childhood Autism Rating Scale. Only children with classical autism and PDD-NOS were included in the study. Age and gender matched 50 healthy children (mean age: 5.6 ± 2.5 years) who were admitted to Erciyes University Children's Hospital Healthy Child Clinic comprised the control group. A questionnaire was applied to parents to determine potential exposure ways to EDCs. The study was approved by Erciyes University's Ethical Committee. Written informed consent was obtained from the parents before participation.

2.2. Preparation of plasma and erythrocyte sample

Venous blood samples were taken by a stainless steel needle from the left arm cubital vein, and the sample was allowed to drop directly into heparinized glass test tubes. The tube openings were covered by clean aluminum foil to protect the sample from contacts with plastic material. Samples were centrifuged immediately at 800*g* for 15 min to obtain plasma and erthyrocyte. Both plasma and erythrocyte samples were aliquoted and kept at −80 °C until analysis.

2.3. Chemicals, kits and equipment

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO). All high performance liquid chromatography (HPLC) equipments were from Agilent (Santa Clara, CA). Colorimetric assay kits for protein determination, glutathione peroxidase (GPx1), thioredoxin reductase (TrxR) and catalase (CAT) were obtained from Sigma-Aldrich (St. Louis, MO). Glucuronidase/aryl sulfatase (from *Helix pomatia*) was also from Sigma-Aldrich. Total superoxide dismutase (SOD), glutathione reductase (GR), total glutathione (GSH) and thiobarbituric acid reactive substance (TBARS) kits were obtained from Cayman Chemical Company (Ann Arbor, MI).

2.4. Measurement of di-(2-ethylhexyl)-phthalate (DEHP) and mono-(2-ethylhexyl)-phthalate (MEHP) levels in plasma

All glass tubes which venous blood samples were taken were heated in an oven at 400 °C for 4 h and all the glassware were washed with n-hexane:tetrahydrofuran (1:1, v/v) for 4 h and than dried in an incubator to after the general cleaning procedure to remove any plastic material residue.

DEHP and MEHP levels was detected by HPLC after extraction from plasma according to Paris et al. (2003) with some modifications. Briefly, 200 μl of plasma was spiked with 20 μl 20 ppm DEHP (1 ppm in the last volume) and 20 μl 20 ppm MEHP (1 ppm in the last volume). After extraction by 400 μl NaOH (1 N), 100 μl%50 H_3PO_4 and 600 μl asetonitrile, samples were vortexed for 1 min. The mixture was centrifugated at 1000*g* for 10 min. The extraction was repeated and supernatants were collected. 900 μl of the supernatant was taken into another tube and evaporated under nitrogen stream. The residues were kept at −20 °C until analysis. Later, residues were dissolved in 400 μl of 60% acetonitrile and 100 μl of these resultants were injected into our HPLC (Hewlett Packard Agilent 1200 Series with Fluorescence Detector, Vienna, Austria). The retention times for DEHP and MEHP were 39.3 min and 4.7 min, respectively. Recovery studies were performed on blank samples of plasma spiked with levels of 7.5 μg/ml of DEHP and 1.25 μg/ml of MEHP, and the average recoveries were found to be (mean ± SD) 93.41 ± 23.41 for DEHP and 82.65 ± 0.97% for MEHP on 10 occasions. Within-day precisions were (% CV) for DEHP 1.12 ± 0.56% and 4.15 ± 1.73% for MEHP. Between-run precisions were 10.31 ± 16.09% coefficient of variation (CV) for DEHP and 8.42 ± 4.42% CV for MEHP. The concentrations of DEHP and MEHP in the samples were calculated from DEHP and MEHP standards and the calibration curve of peak area was used. Limit of detections (LOD) for both DEHP and MEHP were 0.05 μg/ml, and limit of quantifications (LOQ) were for both DEHP and MEHP were 0.1 μg/ml. Determination of DEHP and MEHP concentrations was conducted by HPLC equipped with an auto sampler (Hewlett Packard Agilent 1100 Series, Vienna, Austria) using a UV detector. Spherisorb C18 ODS2 kolon (25 cm × 5 μm × 4.6 mm i.d.) column (Waters, Milford, MA) and ODS C18 precolumn (4 cm) (Waters, Milford, MA) were used for analysis. The mobile phase was 0.1% ortophosphoric acid and acetonitrile [pH 3.0, 80:20 (v/v)], and the flow rate was 1 ml/min.

2.4. Bisphenol a analysis in plasma

BPA was detected by HPLC after extraction from plasma according to Yang et al. (2003). For total BPA (conjugated plus free form), 500 μl plasma sample was spiked with 50 μl 50 ng/ml BPA (5 ng/ml spike in the last volume) and 30 μl of 2.0 M sodium acetate buffer (pH 5.0) was added to the mixture. 10 μl glucuronidase/aryl sulfatase (from *H. pomatia*) was added and mixed. The mixture was incubated 37 °C for 3 h at water bath. After incubation, 100 μl 2 N HCl was added and the mixture was extracted with 5 ml of ethyl acetate. Later, sample was centrifugated at 800*g* for 5 min 3 ml of supernatant was taken to a new glass tube, evaporated under nitrogen stream and the residues were kept at −20 °C until analysis. On the experiment days, the residues were dissolved in 300 μl of 60% acetonitrile and 100 μl of the resultants were injected into our HPLC. HPLC parameters were as follows: C18 col-

umn (25 cm × 5 μm × 4.6 mm i.d.); mobile phase: acetonitrile and 2.5% tetrahydrofuran in water (Gradient elution was performed as 60:40–5:95); flow rate: 0.4 ml/min; column temperature: 25 °C; injection volume: 100 μl; retention time: 18.1 min; excitation wavelength: 230 nm, emission wavelength: 315 nm; analysis time: 40 min. Limit of detection (LOD) was 1 ng/ml and limit of quantitation (LOQ) was 2.5 ng/ml. Recovery studies were performed on blank samples of plasma spiked with levels of BPA, and the average recoveries were found to be (mean ± SD) 97.37 ± 1.23% on ten occasions. Between-run precision was 2.76 ± 0.24% coefficient of variation (CV) and within-day precision was 2.63 ± 1.23% CV. The concentration of BPA in the samples were calculated by using the calibration curve of peak height prepared from BPA standards.

2.5. Determination of oxidant/antioxidant parameters

Throughout the spectrophotometric and spectrofluorometric measurements, SpectraMax M2 (Molecular Devices, Sunnyvale, CA) was used as spectrophotometer and spectrofluorometer. SoftMax Pro Software (Molecular Devices, Sunnyvale, CA) was used for quantification.

The biochemical function of GPx1 is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. This reaction plays a crucial role in protecting cells from damage by free radicals, which are formed by peroxide decomposition. While measuring this activity, the enzyme converts reduced glutathione (GSH) to oxidized glutathione (GSSG). A commercial "glutathione peroxidase assay kit" was used to measure the GPx1 activity in erythrocytes (Günzler et al., 1974; Flohé and Günzler, 1984). The assay relies on the continuous reduction of GSSG (formed during GPx1 reaction) to reduced glutathione (GSH) by glutathione reductase (GR). An organic peroxide, namely t-butyl hydroperoxide, was used as a substrate. The decrease in NADPH absorbance due to its concomitant oxidation to NADP+ was monitored spectrophotometrically at 340 nm. One unit of enzyme was defined as the amount of GPx1 that transformed 1 μmole of NADPH to NADP+ per min at 37 °C.

Thioredoxin reductases are selenoenzymes that reduce thioredoxins (as well as non-disulfide substrates such as selenite, lipoic acids, lipid hydroperoxides, and hydrogen peroxide). These ubiquitous enzymes are involved in many cellular processes such as cell growth, p53 activity, and protection against oxidation stress (Arnér et al., 1999). Erythrocyte TrxR activity was determined using the Thioredoxin Reductase Assay kit. The method was based on the reduction of substrate 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) into 5-thio-2-nitrobenzoic acid (TNB) the concentration of which was measured at 412 nm. As both GPx1, and GR can also contribute to the total reduction of DTNB, their activities can estimated by using a specific TrxR inhibitor. In order to determine the DTNB reduction due only to TrxR activity present in the sample, two assays were performed: in the first measurement, we measured the total DTNB reduction by the sample and in the second measurement, we measured DTNB reduction by the sample in the presence of the TrxR inhibitor solution. The difference between the two results is the DTNB reduction due to TrxR activity. One unit of TrxR activity was defined as the amount of enzyme that provided an increase absorbance of 1.0 per min and per mL at pH 7.0 at 25 °C.

Catalase is an enzyme that is involved in the detoxification of hydrogen peroxide (H_2O_2), which is a toxic metabolite of normal aerobic metabolism. CAT catalyzes the conversion of H_2O_2 to molecular oxygen and water. Activity of CAT was determined colorimetrically using a catalase assay kit. The enzymatic decomposition of H_2O_2 was stopped with a stop solution and the remaining H_2O_2 was detected with a chromogen solution (150 mM potassium phosphate buffer, pH 7.0, containing 0.25 mM 4-aminoantipyrine and 2 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid at 520 nm. One unit of catalase was defined as the enzyme that decomposed one μM of H_2O_2 to oxygen and water per minute at pH 7.0 at 25 °C.

Superoxide dismutases are metalloenzymes that catalase the dismutation of superoxide to H_2O_2 and molecular oxygen and SODs are crucial for prevention of oxidative stress-related conditions and diseases (Kang, 2015). The total SOD activity was measured by a commercial kit which uses cell proliferation reagent (WST-1) that produces a water-soluble formazan dye upon reduction with a superoxide anion. The reduction rate of O_2 was linearly associated with xanthine oxidase (XO) activity and was inhibited by SOD. The IC_{50} (50% inhibition activity of SOD) was determined by this colorimetric method. Since the absorbance at 440 nm was proportional to the amount of superoxide anion, the inhibition of SOD activity was quantified by measuring the decrease in the color development at 440 nm.

GR is a flavoprotein that catalyzes the NADPH-dependent reduction of GSSG to GSH and is essential for the maintaining adequate levels of cellular GSH (Deponte, 2013). The activity of GR was measured by a glutathione reductase assay kit, based on the reduction of GSSG by NADPH in the presence of GR. One unit of enzyme was defined as the enzyme activity that caused the reduction of 1 μmol of DTNB at 25 °C at pH 7.5 (Goldberg and Spooner, 1983). The results are expressed in mU/mg protein.

2.6. Determination of total glutathione levels

Glutathione is an atypic tripeptide that serves as a nucleophlic cosubstrate of glutathione *S*-transferases in the detoxification of xenobiotics. It is also an essential electron donor for GPx enzymes. Total glutathione content was assessed using a total glutathione assay kit based on a kinetic assay. The proteins in the samples were precipitated using metaphosphoric acid and after deproteination, continuous reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) by catalytic amounts of GSH to 5-thio-2-nitrobenzoic acid (TNB) was measured at 405 nm for 25 min (Akerboom and Sies, 1981). Quantification was achieved by parallel measurements of a standard curve of known GSH concentrations, and results were expressed in nmol/mg protein.

2.7. Determination of lipid peroxidation

Lipid peroxidation (LP) is a well-established mechanism in cellular injury and is used as a biomarler of oxidative stress. Lipid peroxidases are unstable compounds that are derived from polyunsaturated faty acids. They can form a series of compounds which include malondialdehyde (MDA). Plasma LP levels were quantified by using a TBARS assay kit which measures the concentration of thiobarbituric acid reactive substance (TBARS) by a spectrofluorometric assay. Measurements were performed at the exitation wavelength of 530 nm and emission wavelength of 550 nm (Richard et al., 1992). Quantification was achieved by parallel measurements of a standard curve of known TBARS concentrations, and results were expressed as nmol/g protein.

2.8. Determination of erythrocyte selenium levels

A single-beam atomic absorption spectrometer (PerkinElmer AAS Spectrometer 700, Waltham, MA) with a Zeeman background correction equipped with an Fs-go plus furnace autosampler was used in the determination of plasma Se levels. A stock solution of 1000 mg/L Na_2SeO_3 was used on a daily basis to prepare appropriate concentrations of standard selenium solutions. Erythrocyte samples (0.2 ml) were diluted with 0.5 ml%0,2HNO_3 (v/v in deionized water) and 0.3 ml%0,2 (v/v in deionized water) Triton X-100. Samples and standard solutions were wet-injected in the graphite

Table 1
Basic characteristics of the study and control groups*.

Characteristics	Control group (n = 50)	Study group (n = 51)
Age (year), mean ± SD	5.6 ± 2.5	5.8 ± 2.5
Male gender, n (%)	39 (78)	41 (80)
Inhabitance, n (%)		
Urban	48 (96)	46 (90)
Rural	2 (4)	5 (10)
Birth order, n (%)		
1	22 (44)	30 (60)
2–3	17 (34)	14 (28)
≥4	11 (22)	7 (14)
Birth weight, mean ± SD (g)	3220 ± 380	3430 ± 725
Gestational age (week), mean ± SD	38 ± 1	38 ± 1
Current weight (kg), mean ± SD	20 ± 6	20 ± 6.2
height (cm), mean ± SD	110 ± 16	108 ± 12
Mother's age (year), mean ± SD	31 ± 6	33 ± 5
Mother working status, n (%)		
Housewife	33 (66)	41 (80)
Working	17 (34)	10 (20)
Father's age (year), mean ± SD	36 ± 6	37 ±8

furnace. Three standard additions and peak height measurements were used for quantification (Kırkbright, 1980).

2.9. Statistical analysis

Statistical analysis was performed by using SPSS 16.0 (SPSS Inc, Chicago, IL). The distribution of the values were analyzed by using the Shapiro-Wilk and Kolmogorov-Smirnov test. Data were expressed as mean ± SD for normal distributions and as median (min-max) for abnormal distributions. The comparison between two parametric values was determined by using Student's t-test. Two nonparametric values were compared by using Mann-Whitney U test and Bonferroni correction. The correlation between nonparametric values was analyzed by using Spearman's correlation. Categorical variables were compared by using chi-square test (χ^2). p values < 0.05 were considered as statistically significant.

3. Results

The study and control groups were similar in terms of age, sex, birth weight, gestational age, birth order, current weight and height, inhabitance, mother's and father's age. Basic characteristics of the patients and healthy controls were shown in Table 1. In the study group, 23,5% (n = 12 males) of the children were diagnosed with PDD-NOS. Children in the study group have no history of precocious puberty, hypospadias or labial adhesion. However, two of the subjects had his

3.1. Questionnaire evaluations

For the evaluation of the questionnare, Chi-square test was used to compare categorical variables. According to the answers obtained from the questionnare (Table 2 and Table 3) teething gum shield usage was significantly higher in the study group compared to control (p = 0.016). Besides, the study subjects were exposed more to polyvinyl chloride (PCV) materials at home (flooring/windows/door frames) than control group (p = 0.014).

The exposure of mothers to EDCs by personal care products and detergents during pregnancy or routinely were shown in Table 3. The number of mothers using make-up was higher in the control group during pregnancy (p = 0001) and currently (p = 0013) compared to study group. Although the rate of detergent usage was not statistically different between the groups (p = 0.06), the rate of using fabric softeners was markedly higher in the study group mothers during both pregnancy (p < 0001) and routinely (p < 0001).

3.2. Plasma DEHP, MEHP, and BPA levels

The mean detectable DEHP, MEHP and BPA levels in both groups were shown in Table 4. The distribution was analyzed by using the Kolmogorov-Smirnov test for DEHP and BPA levels. The distribution was analyzed by using the Shapiro-Wilk test for MEHP levels. Student'st test was used to compare DEHP and MEHP levels and Mann-Whitney U test was used to compare BPA levels between the groups. Because of the limited number of subjects in the PDD-NOS group, a nonparametric test (Kruskal-Wallis one-way analysis of variance test) was used to compare the multiple groups for DEHP and MEHP levels. Mann-Whitney U test with Bon-ferroni correction was used to compare BPA levels between the groups.

Detectable plasma DEHP, MEHP and BPA levels were not significantly different between the study group and the control group. Besides, no marked difference was observed in the detectable plasma DEHP, MEHP and BPA levels between the genders (p = 0.43; p = 0.71; p = 0.67, respectively). However, the median BPA levels of children with PDD-NOS were significantly higher than both classic autistic children and healthy controls (χ^2 = 10.09, p = 0006) (Table 5). Moreover, we evaluated the correlations between the plasma DEHP, MEHP and BPA levels in study and control groups. A significant positive correlation was found between plasma MEHP and BPA levels (Spearman's correlation r = 0.818, p = 0.004) in study group. Plasma DEHP, MEHP or BPA levels were not correlated with children's weight in any of the groups.

Plasma DEHP, MEHP and BPA levels were not significantly different between the children who used teething gum shield or not; between the families that had PVC windows/floorings or not; between children whose mother uses fabric softeners or not (data not shown).

3.3. Oxidant and antioxidant status

For the oxidant and antioxidant parameters, distribution of the carbonyl levels were analyzed by using the Shapiro-Wilk test and the distribution of the other values were analyzed by Kolmogorov-Smirnov test. Student'st test was used to compare normally distributed data, and Mann-Whitney U test was used for the data that is not normally distributed (i.e., selenium and carbonyl levels, and SOD and GR citivities). When comparing PDD-NOS group with control and classic autism groups, because of the limited number of subjects in PDD-NOS group, a nonparametric test (Kruskal-Wallis one-way analysis of variance test) was used to compare the multiple groups. Mann-Whitney *U* test with Bon-ferroni correction was used to compare multiple groups for selenium levels, and GPx1, CAT, SOD, and GR activities.

Plasma TBARS and carbonyl levels, erthyrocyte GPx1, TrxR, CAT, SOD and GR activities and erthyrocyte selenium and GSH levels were shown in Table 6. Carbonyl levels were significantly higher in the study group than controls (p = 0.025). Erthyrocyte selenium levels, GPx1, SOD and GR activities were higher (p = 0.013; p = 0.002; p = 0.03; p < 0.001, respectively) and CAT activity was lower (p < 0.001) in the autistic group compared to control. There were no significant differences in plasma TBARS levels, erythrocyte GSH content and TrxR activity between the groups (p = 0.06; p = 0.87; p = 0.18, respectively).

Table 7 shows oxidant/antioxidant parameters in classic autism, PDD-NOS and control groups. There were no differences between the children with PDD-NOS and classic autism in any of the parameters measured. Correlations between DEHP, MEHP, BPA levels and oxidant-antioxidant parameters were evaluated and there were no significant correlations in the study group (data not shown).

Table 2
Potential Exposure Routes of Children to Endocrine Disrupting Chemicals.

Potential ways	Controls (n = 50)	Patients (n = 51)	p	Potential ways	Controls (n = 50)	Patients (n = 51)	p
Breasfeeding				Plastic plate/spoon			
Yes	47 (94)	49 (96)	0.678	Yes	1 (2)	5 (10)	0.205
No	3 (6)	2 (4)		No	49 (98)	46 (90)	
Duration of breastfeeding				Plastic food container			
≤6 months (n = 21)	13 (28)	8 (16)	0.179	Yes	30 (60)	29 (57)	0.749
>6 months (n = 75)	34 (72)	41 (84)		No	20 (40)	22 (43)	
Baby bottle usage				Drinking water in plastic bottle			
Yes	34 (68)	35 (69)	0.946	Yes	43 (86)	41 (80)	0.479
No	16 (32)	16 (31)		No	7 (14)	10 (20)	
Pacifier usage				Foods covered with plastic			
Yes	14 (28)	15 (29)	0.875	Yes	40 (80)	41 (80)	0.961
No	36 (72)	36 (71)		No	10 (20)	10 (20)	
Plastic toys				Deep-frozen foods			
Yes	21(42)	26 (51)	0.167	Yes	21 (42)	24 (47)	0.609
No	3 (6)	7 (14)		No	29 (58)	27 (53)	
Mix	26 (52)	18 (35)					
Baby shampoo				Canned food			
Yes	49 (98)	45 (88)	0.112	Yes	3 (6)	9 (18)	0.122
No	1 (2)	6 (12)		No	47 (94)	42 (82)	
Baby oil				Drinks in plastic bottle			
Yes	21 (42)	25 (49)	0.479	Yes	43 (86)	41 (80)	0.479
No	29 (58)	26 (51)		No	7 (14)	10 (20)	
Teething gum shield				Yoghurt in plastic container			
Yes	8 (16)	19 (37)	0.016	Yes	36 (72)	34 (67)	0.561
No	42 (84)	32 (63)		No	14 (28)	17 (33)	
Hospitalization in intensive care				Candy covered with plastic			
Yes	0	1 (2)	1.000	Yes	46 (92)	42 (82)	0.234
No	50 (100)	50 (98)		No	4 (8)	9 (18)	
Mechanic ventilation		0	–	Chips in plastic package			
Yes	0	0		Yes	35 (70)	36 (71)	0.926
No	50 (100)	51 (100)		No	15 (30)	15 (29)	
Dialysis by peritoneum or hemodialysis				Fish consumption			
Yes	0	0	–	Yes	36 (72)	31 (60)	0.177
No	50 (100)	51 (100)		No	14 (28)	20 (40)	
Operation or any surgical procedure				PVC at home (flooring/windows/door frames)			
Yes	3 (6)	2 (4)	0.678	Yes	10 (20)	24 (47)	0.014
No	47 (94)	49 (96)		No	40 (80)	27 (53)	
Blood transfusion				Smoking at home			
Yes	1 (2)	1 (2)	1.000	Yes	17 (34)	20 (40)	0.586
No	49 (98)	50 (98)		No	33 (66)	31 (60)	
				Smoking mother			
				Yes	4 (8)	7 (14)	0.525
				No	46 (92)	44 (86)	

$p < 0.05$ is considered as statistically significant.

- Chi-square test were used to compare categorical variables.

4. Discussion

Animal studies and limited number of human studies have reported an association between phthalates, BPA and neurodevelopmental disorders (Fox et al., 2010; Kim et al., 2011; Whyatt et al., 2012; Téllez-Rojo et al., 2013; Engel et al., 2010; Swan et al., 2010; Miodovnik et al., 2011; Kim et al., 2009; Braun et al., 2009, 2011; Perera et al., 2012; Stein et al., 2015; Testa et al., 2012; Kaur et al., 2014; Smith et al., 2011; Luo et al., 2013; Wolstenholme et al., 2012; Mathisen et al., 2013). One of the purposes of the current study was to measure plasma levels of most abundant plasticizers (i.e., BPA, DEHP and its main metabolite MEHP) in children with autism. Some studies reported that exposure to these plasticizers might increase oxidative stress (Kaur et al., 2014) and deficient antioxidant capacity was also associated with their toxic effects (Erkekoglu et al., 2014). As oxidative stress is an important factor in the emerging and progression of neurodevelopmental diseases (Chauhan et al., 2004; Zoroglu et al., 2004; Ming et al., 2005; Meguid et al., 2011), we also aimed to determine oxidant/antioxidant status of autistic children in the current work.

Scientists suggest that ASD disorders are consequences of mainly prenatal insults to the developing central nervous system (CNS). In fetal tissues, the cell proliferation, differentiation and apoptosis are regulated by hormones. Fetal nutrient and oxygen supply, which are modified by different types of hormones, affects fetal growth (Fowden and Forhead, 2009). Moreover, brain development is regulated and influenced by different hormones. Particularly, thyroid hormones have big impact on normal embryonal and fetal neurogenesis and there are reports showing that EDCs, specifically phthalates disrupt either synthesis or circulatory

Table 3
Mother's exposure to endocrine disrupting chemicals by personal care products and detergents in the study and control groups.

	Mother's exposure in any time			Mother's exposure during pregnancy		
	Controls (n = 50)	Patients (n = 51)	p	Controls (n = 50)	Patients (n = 51)	p
Hair dye						
Yes	14 (28)	16 (31)	0711	4 (8)	2 (4)	0.436
No	36 (72)	35 (69)		46 (92)	49 (96)	
Make-up						
Yes	30 (60)	18 (35)	0013	25 (50)	10 (20)	0.001
No	20 (40)	33 (65)		25 (50)	41 (80)	
Nail polish						
Yes	4 (8)	6 (12)	0741	4 (8)	3 (6)	0.715
No	46 (92)	45 (88)		46 (92)	48 (94)	
Shower gel						
Yes	6 (12)	7 (14)	0796	2 (4)	6 (12)	0.269
No	44 (88)	44 (86)		48 (96)	45 (88)	
Perfume						
Yes	30 (60)	29 (39)	0749	26 (52)	20 (39)	0.197
No	20 (40)	22 (61)		24 (48)	31 (61)	
Hair spray						
Yes	0	2 (4)	0157	0	0	–
No	50 (100)	49 (96)		50 (100)	51 (100)	
Shampoo						
Yes	49 (98)	49 (96)	1000	49 (98)	46 (90)	0.205
No	1 (2)	2 (4)		1 (2)	5 (10)	
Hair cream						
Yes	26 (52)	17 (33)	0058	26 (52)	17 (33)	0.058
No	24 (48)	34 (67)		24 (48)	34 (67)	
Body gel wash						
Yes	0	0	–	0	0	–
No	50 (100)	51 (100)		50 (100)	51 (100)	
Detergents						
Yes	48 (96)	45 (88)	0269	49 (98)	44 (86)	0.060
No	2 (4)	6 (12)		1 (2)	7 (14)	
Fabric softeners						
Yes	10 (20)	33 (65)	0000	10 (20)	30 (59)	0.000
No	40 (80)	18 (35)		40 (80)	21 (41)	
Dishwashing gloves						
Yes	7 (14)	9 (18)	0616	5 (10)	6 (12)	0.776
No	43 (86)	42 (82)		45 (90)	45 (88)	

$p < 0.05$ is considered as statistically significant.

- Chi-square test were used to compare categorical variables.

Table 4
Detectable plasma di-(2-ethylhexyl)-phthalate (DEHP), mono (2-ethylhexyl)-phthalate (MEHP) and bisphenol A (BPA) levels in the study and control groups.

	Control group (*n*)	Study group (*n*)	*p*
DEHP (μg/ml)	n = 37	n = 31	0.46
Mean ± SD	0.172 ± 0.124	0.198 ± 0.163	
MEHP (μg/ml)	n = 25	n = 17	0.66
Median (min-max)	0.088 (0.050–0.144)	0.068 (0.052–0.151)	
BPA (ng/ml)	n = 35	n = 37	0.19
Median (min-max)	1.12 (0.02–12.44)	2.15 (0.03–13.63)	

Results are given as mean ± SD.
BPA: bisphenol A; DEHP: di-(2-ethylhexyl)-phthalate; MEHP: mono (2-ethylhexyl)-phthalate.

- The distribution was analyzed by using the Kolmogorov-Smirnov test for DEHP and BPA.
- The distribution was analyzed by using the Shapiro-Wilk test for MEHP.
- Student's t-test was used to compare DEHP and MEHP levels and Mann-Whitney U test was used to compare BPA levels.

levels of thyroid hormones (Patel et al., 2011). Skinner et al. (2008) showed that *in utero* exposure of rats to vinclozolin, a well-known endocrine disruptor, caused alterations in the expression of various genes both in the hippocampus and the amygdala. These changes were observed for three generations, though there was no ongoing exposure for the second and third generations. Therefore, disruption of fuctions of different hormones during prenatal period may have significant effects and many consequences on brain and neurodevelopment for multiple generations through both genetic and epigenetic pathways. There are studies in literature that report the potential relationship between autism and environmental exposures (Miodovnik et al., 2011; McCanlies et al., 2012; Braun et al., 2014; Kim et al., 2010; Larsson et al., 2009). Therefore, a questionnare was applied to the parents of the control and autistic children to better understand the possible environmental exposure routes to phthalates and BPA in the first step of the study. Mothers of children with autism reported higher frequency of fabric softener usage in both preconceptional and intrauterine periods. Teething gum shield usage rate and PVC material exposure at home were significantly higher in autistic children than controls. Simi-

Table 5
Detectable plasma di-(2-ethylhexyl)-phthalate (DEHP), mono (2-ethylhexyl)-phthalate (MEHP) and bisphenol A (BPA) levels in the autism, PDD-NOS, and control groups.

	Control (*n*)	Classic autism (*n*)	PDD-NOS (*n*)	*p*
DEHP (μg/ml)	n = 37	n = 25	n = 6	0.308
Mean± SD	0.173 ± 0.12	0.190 ± 0.18	0.233 ± 0.11	
Median (min-max)	0.171 (0.026–0.617)	0.138 (0.044–0.765)	0.274 (0.045–0.317)	
MEHP (μg/ml)	n = 25	n = 13	n = 4	0.091
Mean ± SD	0.089 ± 0.03	0.077 ± 0.03	0.118 ± 0.04	
Median (min-max)	0.088 (0.050–0.144)	0.065 (0.052–0.134)	0.127 (0.068–0.151)	
BPA (ng/ml)	n = 35[c]	n = 27[a]	n = 10[b]	0.006
Mean± SD	2,39 ± 3,13	2.17 ± 2.77	6.91 ± 4,70	
Median (min-max)	1.12 (0.02–12.44)	1.11 (0.03–12.76)	5.89 (0.50–13.63)	

Results are given as mean ± SD.
BPA: bisphenol A; DEHP: di-(2-ethylhexyl)-phthalate; MEHP: mono (2-ethylhexyl)-phthalate.
a/b; p = 0.003, a/c; p = 0.959, b/c; p = 0.003.

- Because of the limited number of subjects in PDD-NOS group, a nonparametric test (Kruskal-Wallis one-way analysis of variance test) was used to compare the multiple groups.

Table 6
Oxidant and antioxidant parameters in the control and study groups.

	Control group (n)	Study group (n)	p
TBARS (umol/mg protein)	n = 45 9.65 ± 2.3	n = 47 10.84 ± 3.6	0.06
Carbonyl groups (nmol/mg protein)	n = 27 0.40 ± 0.09	n = 21 0.52 ± 0.18	0025
GSH (nmol/g Hb)	n = 49 14.24 ± 2.1	n = 51 14.30 ± 1.6	0.87
Selenium[a] (μg/L)	n = 48 93.81 ± 32.1	n = 48 115.50 ± 43.4	0.013
GPx1 (U/mg Hb)	n = 50 24,68 ± 5,2	n = 51 28.18 ± 5.6	0.002
TrxR[a] (mU/mg protein)	n = 46 63.66 ± 21.1	n = 45 57.77 ± 20.8	0.18
CAT (nmol/min/mg Hb)	n = 49 124.78 ± 28.6	n = 49 96.99 ± 37.8	<0.001
SOD[a] (mU/Hb)	n = 43 52.73 ± 34.1	n = 44 67.56 ± 27.7	0.03
GR[a] (U/g Hb)	n = 48 0.10 ± 0.04	n = 50 0.21 ± 0.1	<0.001

Results are expressed as mean ± SD.
CAT: catalase; GPx1: Glutathione peroxidase 1; GR: glutathione reductase; GSH: glutathione.
SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substance; TrxR: thioredoxin reductase.
[a] logarithmic transformed data.

Table 7
Oxidant and antioxidant parameters in children with autism, PDD-NOS and controls.

	Controls	Classic autism	PDD-NOS	p
TBARS (umol/mg protein)	n = 45 9.65 ± 2.3	n = 37 11.15 ± 3.3	n = 10 9.64 (3.63–18.61)	0.15
Carbonyl groups (nmol/mg protein)	n = 27 0.40 ± 0.09	n = 17 0.46 (0.33–0.90)	n = 4 0.59 (0.24–0.79)	0.07
GSH (nmol/g Hb)	n = 49 14.24 ± 2.1	n = 39 14.37 ± 1.6	n = 12 13.64 (12.10–16.95)	0.53
Selenium* (μg/L)	n = 48 93.81 ± 32.1	n = 37 120.23 ± 45.1	n = 11 86.46 (57.76–157.70)	0.04
GPx1 (U/mg Hb)	n = 50 24,68 ± 5.2	n = 39 28.32 ± 5.7	n = 12 28.28 (20.38–37.45)	0.01
TrxR* (mU/mg Hb)	n = 46 63.66 ± 21.1	n = 34 57.4 ± 22.2	n = 11 60.25 (33.83–82.14)	0.25
CAT (nmol/min/mg Hb)	n = 49[c] 124.78 ± 28.6	n = 37[a] 98.7 ± 34.9	n = 12[b] 76.24 (40.17–187.89)	<0.001
SOD* (mU/mg Hb)	n = 43 52.73 ± 34.1	n = 33 67.5 ± 26.7	n = 11 73.99 (16.61–124.51)	0.04
GR* (U/g Hb)	n = 48[z] 0.10 ± 0.04	n = 38[x] 0.21 ± 0.1	n = 12[y] 0.18 (0.07–0.43)	<0.001

CAT: catalase; GPx1: Glutathione peroxidase 1; GR: glutathione reductase; GSH: glutathione.
SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substance; PDD: Pervasive Developmental Disorder-Not Otherwise Specified; TrxR: thioredoxin reductase.
a/b; p = 0.352, a/c; p = 0.001, b/c; p = 0.006, x/y; p = 0.909, x/z: p.
* logarithmic transformed data.

larly, Larsson et al. (2009) reported that maternal exposure to PVC house flooring material resulted in ASD symptoms in their children in five years time. They estimated that indoor PVC floorings can be one of the important sources of airborne phthalate exposure. In another study, Kim et al. (2010) showed that mothers of children with ASD had higher exposure to canned food, plastics, waste incinerators, old electronics, microwavable food and printed fabrics than control group mothers. As these products are known daily exposure sources for phthalates (Braun et al., 2013) and BPA (Rubin, 2011), these findings might support an association between phthalate and BPA exposures and ASDs. However, it was not possible to establish a clear cause-effect relationship by only applying surveys.

In the second step, we compared plasma DEHP, MEHP and BPA levels of the autsitic children with controls; however, they were not statistically different. In a recent study by Testa et al. (2012), the urinary concentrations of oxidized MEHP metabolites were significantly higher in autistic children than healthy controls. The concentration of MEHP was found to be lower when compared to its further oxidized metabolites. In another study, Stein et al. (2013) measured the urinary DEHP metabolites and reported that autistic children had significantly decreased DEHP glucuronidation compared to control group. In addition, the researchers showed that total amount (free plus conjugated) of phthalate metabolites were not different between the groups but free MEHP levels were significantly higher in autistic group. In the present study, we found that plasma BPA levels of the children with PDD-NOS were higher than both classic autistic children and the healthy controls. Plasma DEHP and MEHP levels of children with PDD-NOS were also higher when compared to children with classic autism as well as the controls but the differences were not found to be statistically significant. These results may be related to the limited number of the subjects in the PDD-NOS group. Although children with PDD-NOS do not fully meet criteria for having an autistic disorder, the studies so far have been unable to clearly indicate if there is any difference in

terms of etiology or outcome (Mordre et al., 2012). We might postulate that high plasticizer (i.e., BPA) exposure might be associated with behavioral and social alterations in children with PDD-NOS. More recently, Stein et al. (2015) reported that total urinary BPA levels were higher in autistic children than controls; however, when they excluded 10 children with very high BPA levels, the difference between autistic and control groups was not statistically significant. In another study, Braun et al. (2009) reported that prenatal BPA exposure was found to be associated with externalizing behaviors in 2 year-old-children, especially among females. In animal studies, it was shown that BPA exposure during gestation changed behavior in pairs of interacting juvenile mice, and decreased vasopressin and oxytocin transcripts in embryonic brain (Wolstenholme et al., 2012). In addition, pubertal BPA exposure was shown to increase anxiety like behavior in adulthood and this alteration was associated with decreased acetylcholinesterase activity of the hippocampus in male mice (Luo et al., 2013). Furthermore, prenatal exposure to BPA was shown to disturb the cerebellar morphology in mice offsprings and chicken embryos (Mathisen et al., 2013).

Although it has not been established how phthalates and BPA exert their possible neurotoxic effects, several mechanisms were suggested. Estrogen and testosterone regulate and interact with neurotransmitters; influence the structural and functional organization of the brain (Rubinow and Schmidt, 1996), and gender specific differentiation such as behavior, mood, and socialization (Breedlove, 1994). As phthalates have anti-androgenic and BPA has estrogenic effects, the overall effects of phthalates may particularly be seen on males while BPA might preferentially affect females. However, concerning gender, current literature does not provide a consistent pattern regarding the neurotoxicity of EDCs (Braun et al., 2009; Braun et al., 2011; Perera et al., 2012). In the current study, all children with PDD-NOS were also male. Phthalates and BPA can exert their neurotoxic effects independent from gender through their effects on thyroid hormone regulation (World Health Organization, 2012). Moreover, they may also have an impact on behavior through altering epigenetic pathways (Kundakovic and Champagne, 2011). Combined or repeated exposures to EDCs might have synergistic or additive effects and can cause potentiation of their possible effects on neurodevelopment (Miodovnik et al., 2011; Zhang et al., 2013; National Research Council, 2008). Currently, we determined a positive and strong corelation between plasma MEHP and BPA levels. Similarly, Stein et al. (2015) reported significant correlations between total BPA and MEHP concentrations. This finding may suggest that humans, particularly young children are exposed to the EDCs as mixtures and points out the importance of the combined effects after exposure to these chemicals.

We also evaluated the oxidant/antioxidant status of the autistic children. There are few studies suggesting the oxidant/antioxidant imbalance might be associated with the toxicity of EDCs (Kaur et al., 2014; Erkekoglu et al., 2014). We can report that there was oxidant/antioxidant imbalance in children with autism. We found that several components of the antioxidant system are altered in autism. TBARS levels (indicator of lipid peroxidation) ($p > 0.05$) and carbonyl groups (indicator of protein oxidation) ($p < 0.05$) were higher in autistic group than controls. Increased oxidative stress has been reported in children with ASDs (Chauhan et al., 2004; Zoroglu et al., 2004; Ming et al., 2005; Meguid et al., 2011). Many of these studies showed that plasma malonyldialdehyde (MDA) contents were significantly higher in autistic children compared to non-autistic siblings; indicating that lipid peroxidation is increased in autism. Protein oxidation is also suggested to be an underlying factor in many neurological disorders (Korolainen and Pirttilä, 2009) and might also be a trigger for autism. On the other hand, lipid peroxidation by-products can attack several cellular targets, like proteins. Carbonyl groups may be introduced into proteins by reactions with MDA or 4-hydroxynonenal. Therefore, we can suggest that protein oxidation might occur as a consequence of lipid peroxidation in autistic children. However, we cannot rule out the possibility that it can also arise independently. On the other hand, GSH levels were reported to be lower in autistic children than controls in many studies (Al-Gadani et al., 2009; Paşca et al., 2009; Al-Yafee et al., 2011; Han et al., 2015). However, in our study it was not different from the healthy controls. Similarly, Al-Gadani et al. (2009) reported that both GPx and SOD activities were significantly higher in autistic group compared to controls. Zoroglu et al. (2004) also found increased SOD activity and decreased CAT activity in autistic children. Söğüt et al. (2003) reported unchanged plasma SOD activity and increased GPx activity in autism. In contrast, decreased activity of GPx in plasma (Yorbik et al., 2002) and in erythrocytes (Yorbik et al., 2002; Paşca et al., 2006), and decreased SOD (Söğüt et al., 2003) activity in erythrocytes were also reported. We showed that autistic children had higher erythrocyte selenium levels than controls, although available data suggests lower (Jory and McGinnis 2008) or similar (Adams et al., 2011) selenium levels. In our study, we observed that CAT activity was lower and SOD and GR activities were higher in autistic group than control, suggesting an imbalance toward cellular oxidation state. However, oxidant/antioxidant biomarkers were not statistically different between children with classic autism and PDD-NOS, except the significant decreases in CAT activity and selenium levels and unsignificant increase in protein oxidation (28%).

To our knowledge, there is no comprehensive study in literature investigating the relationship between EDC exposure, oxidative stress and autism. However, in this case-control study, we did not detect any significant correlations between DEHP, MEHP, BPA levels and oxidant-antioxidant parameters in the study group. Our findings herein, suggest increased oxidative stress and an adaptive response in antioxidant defense system and this can lead to alterations in some antioxidant parameters (i.e., CAT, SOD activities, protein oxidation levels) in autistic children.

There are some limitations of this study. When we designed the study, we prefered to measure plasma levels of DEHP, MEHP and BPA as it was not possible to obtain urine samples from autistic children without any contact to plastic material. However, there are some concerns about the using blood samples instead of urine in literature. In addition, we did not obtain any samples from mothers when they were pregnant or any blood/cord blood sample from the children when they were born. Moreover, this study was conducted on limited number of subjects although we included sufficient number of children to perform statistical analyses. Besides, the number of children with PDD-NOS was also insufficient. We can suggest that longitudinal studies are needed in which mothers and children are followed from preconception and intrauterine to postnatal periods in order to evaluate the association between phthalate and BPA exposures and autism.

In conclusion, this study can suggest that exposure to EDCs (like phthalates and BPA) might be a triggering factor in the outcome of neurodevelopment disorders like PDD-NOS. In this study, we showed that children with PDD-NOS have higher BPA levels compared to classic autistic children. Our findings also support that autistic children have increased oxidative stress as evident by changes in cellular oxidant/antioxidant status. Prospective studies with higher number of the children are needed to determine whether exposure to plasticizers and alterations in oxidant/antioxidant balance are underlying factors of autism, especially for PDD-NOS.

Conflict of interest

The authors declare conflicts of interest.

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References

Adams, J.B., Audhya, T., McDonough-Means, S., Rubin, R.A., Quig, D., Geis, E., et al., 2011. Nutritional and metabolic status of children with autism vs. neurotypical children, and the association with autism severity. Nutr. Metab. (Lond.) 8, 34.
Akerboom, T.P., Sies, H., 1981. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol. 77, 373–382.
Al-Gadani, Y., El-Ansary, A., Attas, O., Al-Ayadhi, L., 2009. Metabolic biomarkers related to oxidative stress and antioxidant status in Saudi autistic children. Clin. Biochem. 42, 1032–1040.
Al-Yafee, Y.A., Al-Ayadhi, L.Y., Haq, S.H., El-Ansary, A.K., 2011. Novel metabolic biomarkers related to sulfur-dependent detoxification pathways in autistic patients of Saudi Arabia. BMC Neurol. 11, 139.
American Psychiatric Association (APA), 2013. Diagnostic and statistical manual of mental disorders, 5th edition. American Psychiatric Publishing : Arlington, VA, pp. 5–25.
Arnér, E.S., Zhong, L., Holmgren, A., 1999. Preparation and assay of mammalian thioredoxin and thioredoxin reductase. Methods Enzymol. 300, 226–239.
Braun, J.M., Yolton, K., Dietrich, K.N., Hornung, R., Ye, X., Calafat, A.M., et al., 2009. Prenatal bisphenol A exposure and early childhood behavior. Environ. Health Perspect. 117, 1945–1952.
Braun, J.M., Kalkbrenner, A.E., Calafat, A.M., Yolton, K., Ye, X., Dietrich, K.N., et al., 2011. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics 28, 873–882.
Braun, J.M., Sathyanarayana, S., Hauser, R., 2013. Phthalate exposure and children's health. Curr. Opin. Pediatr. 25, ç247–ç254.
Braun, J.M., Kalkbrenner, A.E., Just, A.C., Yolton, K., Calafat, A.M., Sjödin, A., et al., 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and old children: the HOME study. Environ. Health Perspect. 122, 513–520.
Breedlove, S.M., 1994. Sexual differentiation of the human nervous system. Annu. Rev. Psychol. 45, 389–418.
Centers for Disease Control and Prevention, 2011. Prevalence of Autism Spectrum Disorders Among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2010. MMWR 63(No. SS02): 1–21.
Chauhan, A., Chauhan, V., Brown, W.T., Cohen, I., 2004. Oxidative stress in autism: increased lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin–the antioxidant proteins. Life Sci. 75, 2539–2549.
Colborn, T., 2004. Neurodevelopment and endocrine disruption. Environ. Health Perspect 112, 944–949.
Deponte, M., 2013. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochim. Biophys. Acta 1830, 3217–3266.
Engel, S.M., Miodovnik, A., Canfield, R.L., Zhu, C., Silva, M.J., Calafat, A.M., et al., 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ. Health Perspect. 118, 565–571.
Erkekoglu, P., Zeybek, N.D., Giray, B.K., Rachidi, W., Kızılgün, M., Hininger-Favier, I., et al., 2014. The effects of di(2-ethylhexyl) phthalate on rat liver in relation to selenium status. Int. J. Exp. Pathol. 95, 64–77.
Flohé, L., Günzler, W.A., 1984. Assays of glutathione peroxidase. Methods Enzymol. 105, 114–121.
Fowden, A.L., Forhead, A.J., 2009. Endocrine regulation of feto-placental growth. Horm. Res. 72, 257–265.
Fox, D.A., Opanashuk, L., Zharkovsky, A., Weiss, B., 2010. Gene-chemical interactions in the developing mammalian nervous system: effects on proliferation, neurogenesis and differentiation. Neurotoxicology 31, 589–597.
Günzler, W.A., Kremers, H., Flohé, L., 1974. An improved coupled test procedure for glutathione peroxidase (EC 1-11-1-9-) in blood. Z. Klin. Chem. Klin. Biochem. 12, 444–448.
Goldberg, D.M., Spooner, R.J., 1983. Glutathione reductase. In: Bergmeyer, H.U., Bergmeyer, J., Grabl, M. (Eds.), Methods of Enzymatic Analysis, vol. 111, 3rd edition. Verlag Chemie Weinheim, pp. 258–265.
Grandjean, P., Landrigan, P.J., 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368, 2167–2178.
Han, Y., Xi, Q.Q., Dai, W., Yang, S.H., Gao, L., Su, Y.Y., et al., 2015. Abnormal transsulfuration metabolism and reduced antioxidant capacity in Chinese children with autism spectrum disorders. Int. J. Dev. Neurosci. 46, 27–32.
Jory, J., McGinnis, W., 2008. Red-Cell trace minerals in children with autism. Am. J. Biochem. Biotech. 4, 101–104.
Kırkbright G.E. 1980. Atomic absorption spectroscopy. In: Elemental analysis of biological materials. Vienna Technical Report Series. 197. International Atomic Agency, Vienna, IAEA, pp. 141–165.
Kang, S.W., 2015. Superoxide dismutase 2 gene and cancer risk: evidence from an updated meta-analysis. Int. J. Clin. Exp. Med. 8, 14647–14655.
Kaur, K., Chauhan, V., Gu, F., Chauhan, A., 2014. Bisphenol A induces oxidative stress and mitochondrial dysfunction in lymphoblasts from children with autism and unaffected siblings. Free Radic. Biol. Med. 76, 25–33.
Kim, B.N., Cho, S.C., Kim, Y., Shin, M.S., Yoo, H.J., Kim, J.W., et al., 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol. Psychiatry 66, 958–963.
Kim, S.M., Han, D.H., Lyoo, H.S., Min, K.J., Kim, K.H., Renshaw, P., 2010. Exposure to environmental toxins in mothers of children with autism spectrum disorder. Psychiatry Investig. 7, 122–127.
Kim, Y., Ha, E.H., Kim, E.J., Park, H., Ha, M., Kim, J.H., et al., 2011. Prenatal exposure to phthalates and infant development at 6 months: prospective mothers and children's environmental health (MOCEH) study. Environ. Health Perspect. 119, 1495–1500.
Korolainen, M.A., Pirttilä, T., 2009. Cerebrospinal fluid, serum and plasma protein oxidation in Alzheimer's disease. Acta Neurol. Scand. 119, 32–38.
Kundakovic, M., Champagne, F.A., 2011. Epigenetic perspective on the developmental effects of bisphenol A. Brain Behav. Immun. 25, 1084–1093.
Larsson, M., Weiss, B., Janson, S., Sundell, J., Bornehag, C.G., 2009. Associations between indoor environmental factors and parental-reported autistic spectrum disorders in children 6–8 years of age. Neurotoxicology 30, 822–831.
Luo, G., Wei, R., Niu, R., Wang, C., Wang, J., 2013. Pubertal exposure to Bisphenol A increases anxiety-like behavior and decreases acetylcholinesterase activity of hippocampus in adult male mice. Food Chem. Toxicol. 60, 177–180.
Mathisen, G.H., Yazdani, M., Rakkestad, K.E., Aden, P.K., Bodin, J., Samuelsen, M., et al., 2013. Prenatal exposure to bisphenol A interferes with the development of cerebellar granule neurons in mice and chicken. Int. J. Dev. Neurosci. 31, 762–769.
McCanlies, E.C., Fekedulegn, D., Mnatsakanova, A., Burchfiel, C.M., Sanderson, W.T., Charles, L.E., et al., 2012. Parental occupational exposures and autism spectrum disorder. J. Autism Dev. Disord. 42, 2323–2334.
Meguid, N.A., Dardir, A.A., Abdel-Raouf, E.R., Hashish, A., 2011. Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation. Biol. Trace Elem. Res. 143, 58–65.
Ming, X., Stein, T.P., Brimacombe, M., Johnson, W.G., Lambert, G.H., Wagner, G.C., 2005. Increased excretion of a lipid peroxidation biomarker in autism: prostaglandins Leukot. Essent. Fatty Acids 73, 379–384.
Miodovnik, A., Engel, S.M., Zhu, C., Ye, X., Soorya, L.V., Silva, M.J., et al., 2011. Endocrine disruptors and childhood social impairment. Neurotoxicology 32, 261–267.
Mordre, M., Groholt, B., Knudsen, A.K., Sponheim, E., Mykletun, A., Myhre, A.M., 2012. Is long-term prognosis for pervasive developmental disorder not otherwise specified different from prognosis for autistic disorder? Findings from a 30-year follow-up study. J. Autism Dev. Disord. 42, 920–928.
National Research Council (NRC), 2008. Phthalates and cumulative risk assessment: the tasks ahead. In: Committee on the Health Risks of Phthalates. The National Academies Press, Washington, DC, pp. 21–38.
Paşca, S.P., Nemeş, B., Vlase, L., Gagyi, C.E., Dronca, E., Miu, A.C., et al., 2006. High levels of homocysteine and low serum paraoxonase 1 arylesterase activity in children with autism. Life Sci. 78, 2244–2248.
Paşca, S.P., Dronca, E., Kaucsár, T., Craciun, E.C., Endreffy, E., Ferencz, B.K., et al., 2009. One carbon metabolism disturbances and the C677T MTHFR gene polymprhism in children with autism stpectrum disorders. J. Cell Mol. Med. 13, 4229–4238.
Paris, I., Ruggieri, F., Mazzeo, P., Carlucci, G., 2003. Simultaneous determination of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl)-phthalate in human plasma by high-performance liquid chromatography. Anal. Lett. 36, 2649–2658.
Patel, J., Landers, K., Li, H., Mortimer, R.H., Richard, K., 2011. Thyroid hormones and fetal neurological development. J. Endocrinol. 209, 1–8.
Perera, F., Vishnevetsky, J., Herbstman, J.B., Calafat, A.M., Xiong, W., Rauh, V., et al., 2012. Prenatal bisphenol an exposure and child behavior in an inner-city cohort. Environ. Health Perspect. 120, 1190–1194.
Richard, M.J., Portal, B., Meo, J., Coudray, C., Hadjian, A., Favier, A., 1992. Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin. Chem. 38, 704–709.
Rubin, B.S., 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J. Steroid Biochem. Mol. Biol. 127, 27–34.
Rubinow, D.R., Schmidt, P.J., 1996. Androgens, brain, and behavior. Am. J. Psychiatry 153, 974–984.
Söğüt, S., Zoroğlu, S.S., Ozyurt, H., Yilmaz, H.R., Ozuğurlu, F., Sivasli, E., et al., 2003. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. Clin. Chim. Acta. 331, 111–117.
Skinner, M.K., Anway, M.D., Savenkova, M.I., Gore, A.C., Crews, D., 2008. Transgenerational epigenetic programming of the brain tran-scriptome and anxiety behavior. PLoS One 3, e3745.
Smith, C.A., Macdonald, A., Holahan, M.R., 2011. Acute postnatal exposure to di(2-ethylhexyl) phthalate adversely impacts hippocampal development in the male rat. Neuroscience 193, 100–108.
Stein, T., Schluter, M.D., Steer, R.A., Ming, X., 2013. Autism and phthalate metabolite glucuronidation. J. Autism Dev. Disord. 43, 2677–2685.
Stein, T.P., Schluter, M.D., Steer, R.A., Guo, L., Ming, X., 2015. Bisphenol in children with autism spectrum disorders. Autism Res. 8, 272–283.
Swan, S.H., Liu, F., Hines, M., Kruse, R.L., Wang, C., Redmon, J.B., et al., 2010. Prenatal phthalate exposure and reduced masculine play in boys. Int. J. Androl. 33, 259–269.
Téllez-Rojo, M.M., Cantoral, A., Cantonwine, D.E., Schnaas, L., Peterson, K., Hu, H., et al., 2013. Prenatal urinary phthalate metabolites levels and

neurodevelopment in children at two and three years of age. Sci. Total Environ. 461–462, 386–390.
Tareen, R.S., Kamboj, M.K., 2012. Role of endocrine factors in autistic spectrum disorders. Pediatr. Clin. North Am. 59, 75–88.
Testa, C., Nuti, F., Hayek, J., De Felice, C., Chelli, M., Rovero, P., et al., 2012. Di-(2-ethylhexyl) phthalate and autism spectrum disorders. A.S.N. Neuro. 4, 223–229.
Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., et al., 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ. Health Perspect. 120, 290–295.
Wolstenholme, J.T., Edwards, M., Shetty, S.R., Gatewood, J.D., Taylor, J.A., Rissman, E.F., et al., 2012. Gestational exposure to bisphenol a produces transgenerational changes in behaviors and gene expression. Endocrinology. 153, 3828–3838.
World Health Organization, 2012. Endocrine Disrupters and Child Health Possible Developmental Early Effects of Endocrine Disrupters on Child Health. Available: www.who.int/iris/bitstream/10665/75342/1/9789241503761_eng.pdf. (accessed 15.10.15.).
Yang, M., Kim, S.Y., Lee, S.M., Chang, S.S., Kawamoto, T., Jang, J.Y., et al., 2003. Biological monitoring of bisphenol a in a Korean population. Arch. Environ. Contam. Toxicol. 44, 546–551.
Yorbik, O., Sayal, A., Akay, C., Akbiyik, D.I., Sohmen, T., 2002. Investigation of antioxidant enzymes in children with autistic disorder: prostaglandins Leukot. Essent. Fatty Acids 67, 341–343.
Zhang, W.Z., Yong, L., Jia, X.D., Li, N., Fan, Y.X., 2013. Combined subchronic toxicity of bisphenol A and dibutyl phthalate on male rats. Biomed. Environ. Sci. 26, 63–69.
Zoroglu, S.S., Armutcu, F., Ozen, S., Gurel, A., Sivasli, E., Yetkin, O., 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.