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Systematic Review

Telomere Length and Oxidative Damage in Children and Adolescents with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis

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Abstract

Background: Autism spectrum disorder (ASD) has been reported to confer an increased risk of natural premature death. Telomere erosion caused by oxidative stress is a common consequence in age-related diseases. However, whether telomere length (TL) and oxidative indicators are significantly changed in ASD patients compared with controls remains controversial. The aim of this study was to determine the associations of ASD with TL and oxidative indicators by performing a meta-analysis of all published evidence. **Methods**: The PubMed and Embase databases were searched for articles published up to April, 2024. The effect size was expressed as standardized mean difference (SMD) and 95% confidence interval (CI) via Stata 15.0 software. **Results**: Thirty-nine studies were included. Pooled results showed that compared with controls, children and adolescents with ASD were associated with significantly shorter TL (SMD = –0.48; 95% CI = –0.66– –0.29; $p < 0.001$; particularly in males), lower total antioxidant capacity (TAC: SMD = –1.15; 95% CI = –2.01– –0.30; $p = 0.008$), and higher oxidative DNA (8-hydroxy-2′-deoxyguanosine, 8-OHdG: SMD = 0.63; 95% CI = 0.03–1.23; $p = 0.039$), lipid (hexanolyl-lysine, HEL: SMD = 0.37; 95% CI = 0.13–0.62; $p = 0.003$), and protein (3-nitrotyrosine, 3-NT: SMD = 0.86; 95% CI = 0.21–1.51; $p = 0.01$; dityrosine, DT: SMD = 0.66; 95% CI = 0.521–0.80; $p < 0.01$) damage. There were no significant differences between ASD and controls in 8-isoprostane and oxidative stress index after publication bias correction, and in N-formylkynurenine during overall meta-analysis. **Conclusions**: TL, 8-OHdG, TAC, HEL, 3-NT, and DT represent potential biomarkers for prediction of ASD in children and adolescents.

Keywords: autism; telomere length; oxidative stress; biomarker; meta-analysis

1. Introduction

Autism spectrum disorder (ASD) characterized by impairments in social communication and interaction as well as the presence of limited interests or repetitive, stereotypic behaviors, is a common neurodevelopmental disorder in children and adolescents, with a global prevalence of 0.6% [1]. Children with ASD have been reported at a significantly increased risk for premature mortality compared with controls [2]. Causes of death examination show that the premature mortality of ASD subjects mainly results from natural death, unintentional injury (drowning or traffic accident due to wandering or elopement) and intentional self-harm [3,4]. Subsequently adjusted analysis confirms deaths from natural causes predominate [3]. These findings indicate that ASD may be associated with accelerated biological aging.

Telomeres are the repetitive DNA repeat sequences of 5′-TTAGGG-3′ located at the ends of linear eukaryotic chromosomes to prevent chromosome degradation and maintain genomic stability [5,6]. Telomere length (TL) has been found to be progressively shortened with biological aging, which consequently limits cell proliferation and induces senescence or apoptosis in somatic cells, ultimately promoting the development of aging-related diseases and premature death [7–9]. Theoretically, TL should be shorter in patients with ASD than that in those without. This hypothesis was demonstrated by Zhang *et al.* [10] who detected the absolute quantitative TL was 5042.068 ± 1950.595 kb in the ASD group and 6199.267 ± 2931.947 kb in the typical development (TD) control group ($p = 0.002$). Li *et al.* [11] observed that the relative TL was 0.88 ± 0.28 in patients with ASD and 1.01 ± 0.43 in control ($p = 0.006$). Multivariate analysis also verified a significant correlation between TL shortening and the occurrence of ASD [10,11]. However, Panahi *et al.* [12] and Lewis *et al.* [13] found no significant difference in average TL between ASD and controls (TD or healthy siblings). These inconsistent results point the necessity of re-examining the associations between TL and ASD.

Accumulating evidence has implied that activation of oxidative stress may play a central role in the pathogenesis of ASD [14]. Recently published meta-analyses have identified significant changes in levels of oxidative stress-

related biomarkers for ASD patients: increased pro-oxidant nitric oxide, malondialdehyde and oxidized glutathione; decreased anti-oxidant glutathione and glutathione peroxidase [15,16]. Studies on both WI-38 fibroblasts and HL-60 cells have found that relative to non-telomere sequence, telomere DNA sequence is five times more susceptible to oxidative stress induced by ultraviolet [17,18]. A continuous, exponential correlation between reactive oxygen species (ROS) levels and telomere shortening rates is observed in fibroblasts from human and sheep [19]. Supplementation of antioxidant vitamin E and selenium is reported to slow telomere shortening of free-living white stork chicks [20]. The interaction of ROS with guanines in telomere sequences can result in the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) [17]. Therefore, oxidative stress-induced upregulation of 8-OHdG may represent the mechanisms of telomere attrition in ASD patients. However, contradictory results had been revealed by different studies investigating the levels of 8-OHdG in ASD patients compared with controls: either a significant increase [10,21] or without a statistical difference [22,23].

Here, a comprehensive, systematic review and meta-analysis is designed to analyze the relationships between the risk of ASD and TL, 8-OHdG as well as other oxidative stress-related biomarkers that have not been investigated in published meta-analyses [15,16]. The results may provide potential predictive biomarkers for the early detection and intervention of children and adolescents with ASD to prevent poor prognosis and guide their life trajectory to a more positive path [24].

2. Materials and Methods

2.1 Search Strategy

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [25]. The PRISMA_2020_checklist is included in the **Supplementary Materials-PRISMA_2020_checklist**. Two reviewers independently searched PubMed and Embase databases to identify articles published until April 2024 using the following keywords ("telomere" OR "8-hydroxy-2′-deoxyguanosine" OR "8-OHdG" OR "8-oxo-7,8-dihydro-2′-deoxyguanosine" OR "8-oxoguanine" OR "8-isoprostane" OR "8-iso-PGF2α" OR "total antioxidant capacity" OR "total antioxidant power" OR "total antioxidant status" OR "thiol" OR "disulfide" OR "nitrotyrosine" OR "hexanolyl-lysine" OR "N-formylkynurenine" OR "oxidative stress markers" OR "oxidative stress index") AND ("children" or "childhood" or "adolescents") AND ("autism" OR "autistic disorder"). Searches were not restricted by language. Additionally, the reference lists of selected studies and reviews were manually checked to retrieve potentially eligible literatures.

2.2 Selection Criteria

Studies that met the following criteria were considered eligible: (1) Evaluated the associations between TL, 8-OHdG, oxidative stress biomarkers of interest (8-isoprostane, 8-iso-PGF2α; hexanolyl-lysine, HEL; total antioxidant capacity, TAC; 3-nitrotyrosine, 3-NT; dityrosine, DT; N-formylkynurenine, NFK; oxidative stress index, OSI; thiol/disulfide) and ASD in children and adolescents (defined as $<$22 years based on the degree of individual cognition and socialization [24,26]); (2) Designed as a case (ASD)-control comparative study; (3) Provided sufficient data to estimate effect sizes.

Exclusion criteria included: (1) Duplicate publications; (2) Non-original articles (case reports, reviews, conference abstract or comments); (3) Non-human experimental studies (*in vitro* and *in vivo*); (4) Lack of controls; (5) Biomarkers of interest not measured, measured in less than three studies or data unavailable; (6) Age of partial or all ASD patients $>$22 years; (7) Other irrelevant topics.

2.3 Data Extraction

Two reviewers independently collected the following information from each study: the first author, publication year, country, sample size, average age, gender ratio, diagnostic criteria for ASD, control type, sampling source and data of outcome measures (mean $\pm$ standard deviation). Data were directly obtained from tables/texts or indirectly estimated from figures using the GetData Graph digitizer software (version 2.26; https://getdata-graph-digitizer.software.informer.com/). With regard to any discrepancy in the extracted data, a consensus was reached by thorough discussion with a third reviewer.

2.4 Quality Assessment

The quality of each included article was judged by two authors using the Newcastle–Ottawa Scale (NOS) [27] that consisted of three domains: patient selection (0–4 points), comparability (0–2 points) and outcome (0–3 points). NOS scores ranged from 0 to 9 stars and higher quality studies were defined as NOS scores $>$6.

2.5 Statistical Analysis

The meta-analysis was conducted using Stata software (version 15.0; Stata Corporation, College Station, TX, USA). Pooled effect size was presented as standardized mean difference (SMD) with 95% confidence interval (CI) and determined by Z-test, with a p-value < 0.05 considered statistically significant. The heterogeneity between studies was evaluated using the Cochrane's Q test and I^2 statistic. A value of $p < 0.1$ and $I^2 > 50\%$ indicated substantial heterogeneity between studies and thus a random-effect model was selected to calculate composite results; a fixed-effect model was used when there was no significant heterogeneity ($p > 0.1$ and $I^2 < 50\%$). Subgroup analysis stratified by control type (TD or unaffected sibling), assay unit of TL

[absolute TL in kbp and relative TL based on telomere (T) to single-copy gene (S) sequence (T/S) ratio], sample size (<100 or ≥ 100), race (Asian or non-Asian), sample source (blood, urine, saliva or brain) and detection method for each variable was performed for variables with at least five analyzed data items to explore the potential cause of heterogeneity. Moreover, to further identify potential factors for heterogeneity, a multivariable meta-regression analysis was performed to test the impact of various subgroup covariates on study effect sizes (for variables with at least ten analyzed data items). Egger's linear regression test was utilized to analyze publication bias. If bias was present ($p < 0.05$), the trim-and-fill method was used to correct pooled results. A sensitivity analysis was carried out with the leave-one-out method to explore the effect of each study on the overall result.

3. Results

3.1 Literature Search

Electronic search located 1403 studies, 969 of which were discarded due to duplicate publications. Reading titles and abstracts excluded 390 records as they were case reports ($n = 4$), reviews/meta-analysis ($n = 40$), conference abstract or comments ($n = 11$), animal experiments ($n = 104$), cell experiments ($n = 1$), lack of controls ($n = 5$), adult ASD ($n = 50$) and irrelevant topic ($n = 175$). The full-text of the remaining 44 studies was downloaded and six were removed due to: age of partial ASD patients >22 years ($n = 3$), data unavailable ($n = 1$) and biomarkers detected for mothers of ASD children ($n = 1$). Ultimately, 39 eligible studies were included in this meta-analysis [10–13,21–23,28–59] (Fig. 1).

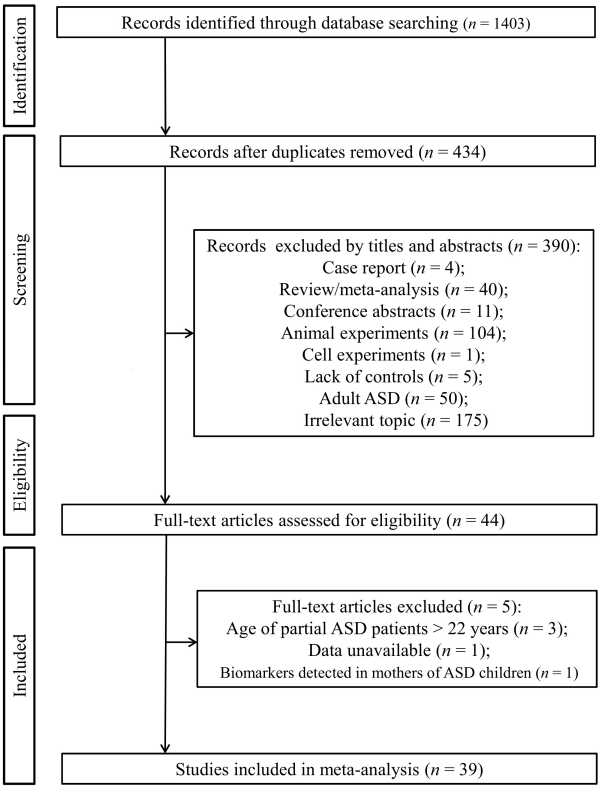

Fig. 1. Flow diagram of study selection for meta-analysis. ASD, Autism spectrum disorder.

3.2 Study Characteristics

Details of all included studies are summarized in Table 1 (Ref. [10–13,21–23,28–59]) and **Supplementary Table 1**. All 39 studies were published in English from 2005 to 2024. Eight studies were undertaken in the USA, four in Egypt, Japan, Saudi Arabia, two in China, India, Iran, Slovenia, Turkey, UK and one in the Belgium, Italy, Nigeria, Qatar, Romania, Slovakia, Spain, respectively. Although the gender ratio of ASD patients varied in different studies, the number of males was typically greater than females in most of the studies. Age was <12 years old (defined as children, which is a stage for the acquisition of brain plasticity and learning capacity) for most participants and 12 to 22 years old in sporadic cases (defined as adolescents, which is the transition period between childhood and adulthood to have the capacity for planning and self-regulation) [22]. ASD patients were diagnosed according to the criteria detailed in the diagnostic and statistical manual of mental disorders (fourth or fifth version), autism diagnostic interview-revised, the autism diagnostic observation schedule, the social communication questionnaire, the childhood autism rating scale, the autism behavior checklist, the developmental, dimensional and diagnostic interview and the international classification of diseases. All studies were high in methodological quality, achieving eight or nine stars.

3.3 Meta-Analysis of the Association of TL with ASD in Children and Adolescents

Six studies [10–13,28,29] with 11 datasets detected TL in ASD patients and controls by polymerase chain reaction assays (**Supplementary Table 1**). Meta-analysis results showed that TL tended to be significantly shortened in patients with ASD compared with controls (SMD = –0.48; 95% CI = –0.66– –0.29; $p < 0.001$) (Table 2; Fig. 2). Subgroup analysis indicated that TL was only significantly shorter in ASD when compared to TD (SMD = –0.49; 95% CI = –0.67– –0.31; $p < 0.001$), but not when compared to unaffected siblings ($p = 0.200$). Subgroup analyses based on TL assay unit, sample size, race and sample source also demonstrated telomere shortening in ASD subjects (**Supplementary Table 2**). Also, the heterogeneity was removed when the sample size was larger than 100. This heterogeneity factor was confirmed in meta-regression analysis (**Supplementary Table 3**): effect size tended to increase with increasing sample size.

Some studies additionally analyzed male or female cases independently or only enrolled males. Therefore, me-

Table 1. Summary of selected qualified studies.

Author	Biomarkers of interest (assay method)	NOS	No.	ASD				Control			Year	Country
				No.	Age (year)	M/F	Diagnosis	No.	Age (year)	M/F		
Zhang T *et al.* [10]	TL (PCR), 8-OHdG (LC–MS/MS), TAC (ELISA)	9	192	96	4.24 ± 1.84	81/15	DSM-V, CARS, ABC	96	4.21 ± 1.05	81/15	2023	China
Panahi Y *et al.* [12]	TL (PCR)	9	58	24	5.13 ± 2.3	14/10	DSM-IV-TR, ADI-R	10 24	6.7 ± 4.62 (sibling) 5.44 ± 2.38 (TD)	8/2 14/10	2023	Iran
Nelson CA *et al.* [28]	TL (PCR)	8	49	18	7.25 (3.5–13.8)	16/12	ADOS, SCQ	31 28	6.75 (4.3–12.1) (TD) 2.17 (0.5–4.42) (sibling)	14/17 16/12	2015	USA
Li Z *et al.* [11]	TL (PCR)	8	239	110	4.75 ± 2.08	98/12	DSM-IV, CARS, ABC	129	4.99 ± 2.17	98/31	2014	China
Lewis CR *et al.* [13]	TL (PCR)	9	155	86	6.4 ± 4.1	86/0	DSM-IV, ADOS	57 69	7.7 ± 4.0 (sibling) 7.1 ± 2.3 (TD)	57/0 69/0	2020	USA
Salem S and Ashaat E [29]	TL (PCR)	9	97	69	6.8 (6–20)	55/14	DSM-V, CARS	28	-	-	2024	Egypt
Sajdel-Sulkowska EM *et al.* [30]	8-OHdG (EIA)	9	11	5	8.9 ± 3.2	5/0	ADI-R	6	9.95 ± 4.73	4/2	2009	USA
Osredkar J *et al.* [31]	8-OHdG, 8-iso-PGF2α, DT, HEL (all ELISA)	8	186	139	10.0 (2.1–18.1)	124/15	DSM-V	47	9.1 (2.5–20.8)	23/24	2019	Slovenia
Ming X *et al.* [32]	8-OHdG, 8-iso-PGF2α (both ELISA)	8	62	33	4–17	29/4	ADI-R, ADOS, DSM-IV	29	5–16	17/12	2005	USA
Osredkar J *et al.* [23]	8-OHdG (LC-MS/MS)	8	211	143	9.5 (2.1–18.1)	126/17	DSM-V	68	8.3 (2.5–20.8)	41/27	2023	Slovenia
Imataka G *et al.* [22]	8-OHdG, TAC, HEL (all ELISA)	8	29	19	10.8 ± 5.2	12/7	ADI-R, DSM-V	10	14.2 ± 7.0	3/7	2021	Japan
El-Ansary A *et al.* [21]	8-OHdG (ELISA)	9	55	28	7.0 ± 2.34	28/0	DSM-IV-TR	27	7.2 ± 2.14	27/0	2018	Saudi Arabia
Ghezzo A *et al.* [33]	8-OHdG, 8-iso-PGF2α, HEL (ELISA), TAC (ORAC)	9	41	21	6.8 ± 2.23	17/4	DSM-IV-TR, ADOS, CARS	20	7.6 ± 1.96	14/6	2013	Italy
Yui K *et al.* [34]	8-OHdG, TAC, HEL (all ELISA)	9	32	20	11.1 ± 5.12	7/13	DSM-V, ADI-R	12	14.3 ± 6.28	4/8	2017	Japan
Yui K *et al.* [35]	8-OHdG, TAC, HEL (all ELISA)	9	31	20	10.7 ± 5.0	7/13	DSM-V, ADI-R, ADOS	11	14.7 ± 6.3	4/7	2017	Japan
Hirayama A *et al.* [36]	8-OHdG (ELISA)	9	97	39	7.7 ± 1.5	32/7	ADI-R	58	7.3 ± 0.95	30/28	2020	Japan
Qasem H *et al.* [37]	8-iso-PGF2α (ELISA)	9	84	44	7 ± 4	-	ADI-R, ADOS, 3DI	40	7 ± 3	-	2016	Saudi Arabia
Mostafa GA *et al.* [38]	8-iso-PGF2α (EIA)	9	88	44	8 (3.5–12)	30/14	DSM-IV	44	8 (4–12)	30/14	2010	Egypt
Pop B *et al.* [39]	8-iso-PGF2α (EIA)	9	48	24	9.02 ± 3.48	-	DSM-IV-TR, ICD10	24	10.11 ± 4.12	-	2017	Romania
Yao Y *et al.* [40]	8-iso-PGF2α (GC-MS)	9	38	26	4.6 ± 0.2	22/4	DSM-IV	12	6.7 ± 0.4	10/2	2006	USA
Omotosho IO *et al.* [41]	TAC (FRAP), OSI	9	50	25	5.96 ± 1.4	-	DSM-IV-TR	25	6.18 ± 2.59	-	2021	Nigeria
Saleem TH *et al.* [42]	TAC (ABTS), OSI	9	118	54	5.74 ± 2.63	46/8	CARS	64	5.42 ± 1.74	49/15	2020	Egypt
Damodaran LPM *et al.* [43]	TAC (ABTS), OSI	9	90	45	4–12	36/9	CARS	45	4–12	36/9	2011	India

Table 1. Continued.

Author	Biomarkers of interest (assay method)	NOS	No.	ASD				Control			Year	Country
				No.	Age (year)	M/F	Diagnosis	No.	Age (year)	M/F		
Rai K *et al.* [44]	TAC (PM)	8	151	101	6–12	-	-	50	6–12	-	2012	India
Ranjbar A *et al.* [45]	TAC (FRAP), thiol	9	53	29	6–12	13/16	DSM-IV-TR	24	6–12	13/11	2014	Iran
Parellada M *et al.* [46]	TAC (ABTS)	9	69	35	12.89 ± 2.58	33/2	DSM-IV, ADOS	35	12.79 ± 2.87	31/3	2012	Spain
Hassan MH *et al.* [47]	TAC (ABTS), OSI	9	146	73	7.13 ± 3.52	73/0	CARS	73	7.76 ± 4.37	73/0	2019	Egypt
Jasenovec T *et al.* [48]	TAC (FRAP)	9	53	36	3.3 (2.7–7.6)	32/4	DSM-V, ADOS-2, ADI-R	17	5.4 (2.4–6.3)	12/5	2023	Slovakia
Ayaydın H *et al.* [49]	Thiol (DTNB)	9	82	42	6.08 ± 2.16	29/13	CARS	40	6.89 ± 2.4	30/10	2021	Turkey
Efe A *et al.* [50]	Thiol (DTNB)	9	116	60	5.89 ± 2.17	54/6	DSM-V	56	6.31 ± 1.57	51/5	2021	Turkey
Ramaekers VT *et al.* [51]	Thiol (DTNB)	9	62	38	7.25 ± 3.9	31/7	ADI-R, ADOS, CARS	24	8.7 ± 3.2	20/4	2020	Belgium
Khan A *et al.* [52]	3-NT (ELISA)	8	21	10	4–15	-	-	11	5–16	-	2014	USA
Anwar A *et al.* [53]	3-NT, DT, NFK (LC-MS/MS)	9	48	27	7.4 ± 2.0	21/6	DSM-IV-TR, ADOS, CARS	21	8.3 ± 2.12	15/6	2016	UK
Nadeem A *et al.* [54]	3-NT (flow cytometry)	9	85	45	7.4 ± 3.25	40/5	DSM-V	40	7.6 ± 2.9	35/5	2019	Saudi Arabia
Sajdel-Sulkowska *et al.* EM [55]	3-NT (ELISA)	9	4	2	11.45 ± 3.75	2/0	ADI-R	2	11.2 ± 4.81	2/0	2011	USA
Frye RE *et al.* [56]	3-NT (LC)	8	90	18 18	8.5 ± 3 7.9 ± 3.2	14/2 15/3	DSM-IV-TR	54	3–10	-	2013	USA
Al-Bishri WM [57]	3-NT (ELISA)	8	50	25	3–11	-	DSM-V, ADOS	25	3–11	-	2023	Saudi Arabia
Anwar A *et al.* [58]	3-NT, DT, NFK (all LC-MS/MS)	9	69	38	7.6 ± 2.0	29/9	DSM-V, ADOS, CARS	31	8.6 ± 2.0	23/8	2018	UK
Al-Saei ANJM *et al.* [59]	3-NT, DT, NFK (all LC-MS/MS)	9	478	311	5.2 ± 3.0	247/64	DSM-V, ADI-R	167	4.9 ± 2.4	94/73	2024	Qatar

ASD, Autism spectrum disorder; TL, telomere length; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HEL, hexanolyl-lysine; TAC, total antioxidant capacity; 8-iso-PGF2α, 8-isoprostane; 3-NT, 3-nitrotyrosine; DT, dityrosine; NFK, N-formylkynurenine; OSI, oxidative stress index; DSM-V, diagnostic and statistical manual of mental disorders; DSM-IV-TR, diagnostic and statistical manual of mental disorders fourth edition, text revised; ADI-R, autism diagnostic interview-revised; ADOS, the autism diagnostic observation schedule; SCQ, the social communication questionnaire; CARS, childhood autism rating scale; ABC, autism behavior checklist; 3DI, the developmental, dimensional and diagnostic interview; ICD, the international classification of diseases; TD, typical development; M, male; F, female; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay methods; LC-MS/MS, liquid chromatography-tandem mass spectrometry; GC-MS, gas chromatography–mass spectrometry; ORAC, oxygen radical absorbance capacity; FRAP, ferric-reducing antioxidant power; ABTS, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) cation bleaching; PM, phosphomolybdate; NOS, Newcastle–Ottawa Scale; PCR, polymerase chain reaction; DTNB, 5,50-dithiobis-(2-nitrobenzoic) acid; LC, liquid chromatography.

ta-analysis for males and females was separated. Meta-analysis of four studies with seven datasets [11–13,28] (**Supplementary Table 1**) showed that male ASD patients possessed a highly significant decrease in TL in comparison to controls (SMD = –0.25; 95% CI = –0.42– –0.09; p = 0.002; particularly for subgroups with TD controls, p = 0.014; Asian population, p = 0.009; saliva samples, p = 0.006) (Table 2). There was no significant difference in TL between female ASD patients and controls regardless of overall (SMD = –0.20; 95% CI = –1.11–0.72; p = 0.670) [12,13,28] or subgroup meta-analyses (**Supplementary Table 2**).

3.4 Meta-Analysis of the Association of Oxidative Stress Biomarkers with ASD in Children and Adolescents

3.4.1 8-OHdG

Eleven studies [10,21–23,30–36] investigated the difference in levels of oxidative DNA damage biomarker 8-OHdG between ASD and controls (**Supplementary Table 1**). Pooled analysis of the data from these studies found that the concentration of 8-OhdG was significantly increased in patients with ASD compared with controls (SMD = 0.63; 95% CI = 0.03–1.23; p = 0.039) (Table 2, Fig. 3), particularly for those with mild-moderate severity [21,23] (SMD = 1.97; 95% CI = 0.32–3.62; p = 0.019) (Table 2).

3.4.2 8-iso-PGF2α

Seven studies [31–33,37–40] compared 8-iso-PGF2α levels in ASD patients with that in healthy controls (**Supplementary Table 1**). Meta-analysis observed a significant increase in levels of 8-iso-PGF2α in the ASD group compared with controls (SMD = 4.61; 95% CI = 2.61–6.60; $p < 0.001$) (Table 2). Subgroup analysis based on sample size, race, sample source and detection method all further demonstrated this significant association (**Supplementary Table 2**). Furthermore, two studies [37,38] with three datasets specifically measured 8-iso-PGF2α levels in ASD patients with mild-moderate or severe impairments. Pooled analysis was independently performed for them. A consistent conclusion was achieved with this analysis and a significantly larger effect size was found (mild-moderate: SMD = 12.11; 95% CI = 7.93–16.30; $p < 0.001$; severe: SMD = 8.48; 95% CI = 4.93–12.03; $p < 0.001$) (Table 2).

3.4.3 TAC

TAC in ASD patients and controls was assessed in 13 studies [10,22,33–35,41–48] with 18 datasets (**Supplementary Table 1**). Random-effects meta-analysis revealed that TAC levels were significantly decreased in ASD patients compared with controls (SMD = –1.15; 95% CI = –2.01– –0.30; p = 0.008) (Table 2). Subgroup analysis showed that significantly lower TAC levels were only detected in urine samples of ASD patients compared with TD controls (**Supplementary Table 2**).

3.4.4 HEL

Five studies [22,31,33–35] provided the HEL levels in ASD patients and controls (**Supplementary Table 1**). Results from meta-analyses indicated that HEL levels were significantly increased in ASD patients compared with controls (SMD = 0.37; 95% CI = 0.13–0.62; p = 0.003) (Table 2). Subgroup analysis showed a significant increase in studies with sample size <100 (p = 0.001) and non-Asian population (p = 0.006) (**Supplementary Table 2**).

3.4.5 OSI

Four studies [41–43,47] with six datasets evaluated changes in OSI between ASD patients and controls (**Supplementary Table 1**). Pooled results observed that OSI in ASD patients was significantly higher than that in controls (SMD = 6.76; 95% CI = 4.20–9.31; $p < 0.001$) (Table 2). Subgroup analysis demonstrated a significant increase in studies with sample size <100 (p = 0.005) and Asian population (p = 0.002) (**Supplementary Table 2**). Meta-analysis of data from ASD patients with mild-moderate (SMD = 14.00) or severe (SMD = 5.76) impairments also indicated a remarkable increase in OSI (Table 2).

3.4.6 Total Thiol

Four independent publications [45,49–51] measured total thiol levels in ASD patients and controls (**Supplementary Table 1**). Meta-analysis found decreased levels of total thiol in ASD patients compared with controls, but the p-value was approximately 0.05 (SMD = –1.65; 95% CI = –3.24– –0.06; p = 0.042) (Table 2), indicating its association with ASD needs further investigation.

3.4.7 3-NT

Eight studies [52–59] involving 29 datasets were included to evaluate the association of 3-NT with ASD (**Supplementary Table 1**). Pooled analysis of these data found that 3-NT was significantly increased in ASD patients compared with controls (SMD = 0.86; 95% CI = 0.21–1.51; p = 0.01) (Table 2). Subgroup analysis showed that 3-NT was significantly increased in the blood samples of ASD patients (p = 0.005), but not significantly changed in brain tissues (p = 0.117) (**Supplementary Table 2**).

3.4.8 DT

Four studies [31,53,58,59] involving six datasets explored the association of DT with ASD (**Supplementary Table 1**). Combined analysis found that DT was significantly increased in ASD patients compared with controls (SMD = 0.66; 95% CI = 0.52–0.80; $p < 0.001$) (Table 2). Subgroup analysis showed that DT residue contents of proteins in blood and urine were both significantly increased ($p < 0.01$). Sample size, race and detection method factors did not change this significant result (**Supplementary Table 2**).

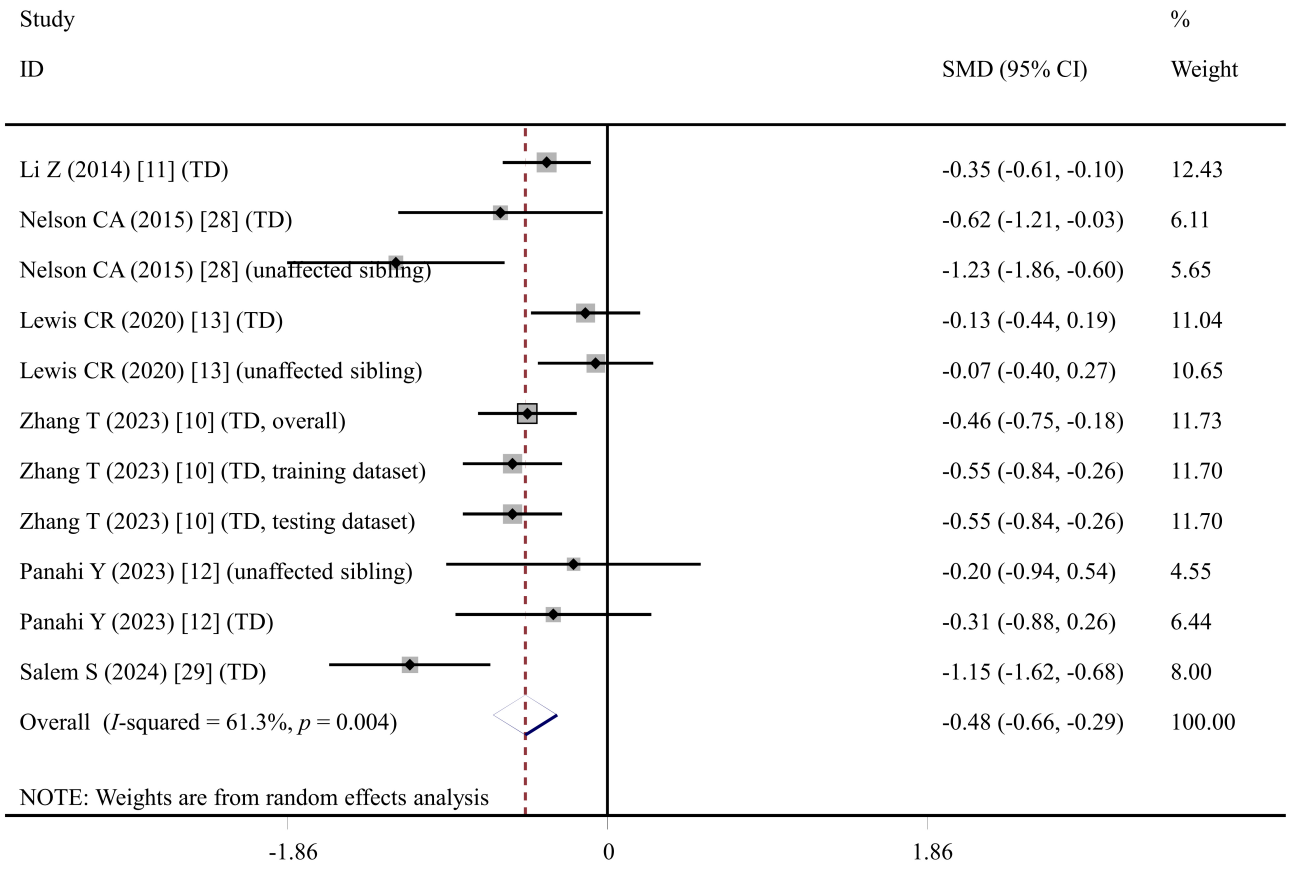

Fig. 2. Forest plots to show the effect size that compared the telomere length in autism patients with that in the control group (TD or unaffected sibling). TD, typical development; SMD, standardized mean difference; CI, confidence interval.

3.4.9 NFK

Three studies [53,58,59] involving five datasets explored the association of NFK, a marker of oxidation in the tryptophan residue of proteins, with ASD (**Supplementary Table 1**). Meta-analysis revealed no significant difference in NFK residue contents of proteins between ASD and control groups ($p = 0.525$). No significant result was obtained in all subgroups with the number of datasets >1 (**Supplementary Table 2**).

3.5 Publication Bias and Sensitivity Analysis

Egger's linear regression test did not find evidence of publication bias for analysis of TL, 8-OHdG, HEL, total thiol, 3-NT and NFK, whereas potential publication bias was observed for 8-iso-PGF2α (all cases, $p = 0.002$; severe cases, $p = 0.044$), TAC (all cases, $p = 0.006$; mild-moderate cases, $p = 0.014$), OSI (all cases, $p = 0.025$; mild-moderate cases, $p = 0.005$) and DT ($p = 0.004$) (Table 2).

The trim-and-fill method was subsequently used to adjust the effect sizes for variables with publication bias. Results showed that although the SMD was slightly changed, the levels of DT (SMD = 0.59; 95% CI = 0.39–0.80; $p < 0.01$) and 8-iso-PGF2α (severe: SMD = 5.39; 95% CI = 1.82–8.96; $p = 0.03$) were still significantly increased in ASD similar to the pre-correction analysis; the SMD and 95% CI were not significantly changed for TAC; 8-iso-PGF2α (all cases, $p = 0.333$) and OSI (all cases, $p = 0.450$; mild-moderate cases, $p = 0.074$) levels in ASD patients were not significantly different from those in controls.

Sensitivity analyses by excluding one study in turn did not find significant changes in the effective estimates for all biomarkers, indicating the robustness of the meta-analysis outcomes (Fig. 4).

4. Discussion

Although there have meta-analyses exploring the association between TL and neurological disorders, they have mainly focused on adult disease, such as schizophrenia [60,61], Alzheimer [62] and Parkinson [63]. To our knowledge, this is the first meta-analysis to integrate all published studies to comprehensively confirm TL changes in children and adolescents with ASD. Consistent with other neurological disorders [60–63], pooled results showed that TL in children and adolescents with ASD was significantly reduced compared with healthy controls (particularly TD). Also, this significant result was not influenced by TL assay unit, sample size, race and sample source. Furthermore, analysis of prevalence studies indicated that the ratio of male to female

Table 2. Meta-analysis of the association of TL and oxidative stress biomarkers with ASD.

Variables	No.	SMD	95% CI	p_E-value	I^2	p_H-value	Model	Egger p (adjusted p_E-value by trim-and-fill)
TL (all cases)	6 (11)	–0.48	–0.66, –0.29	<0.001	61.3	0.004	R	0.329
TL (male cases)	4 (7)	–0.25	–0.42, –0.09	0.002	31.5	0.188	F	0.249
TL (female cases)	3 (5)	–0.20	–1.11, 0.72	0.670	66.9	0.017	R	0.677
8-OHdG (all cases)	11 (11)	0.63	0.03, 1.23	0.039	93.7	<0.001	R	0.165
8-OHdG (mild-moderate cases)	2 (4)	1.97	0.32, 3.62	0.019	96.2	<0.001	R	0.005
8-OHdG (severe cases)	2 (3)	2.69	–0.11, 5.48	0.060	96.4	<0.001	R	0.196
8-iso-PGF2α (all cases)	7 (7)	4.61	2.61, 6.60	<0.001	98.1	<0.001	R	0.002 (0.333)
8-iso-PGF2α (mild-moderate cases)	2 (3)	12.11	7.93, 16.30	<0.001	89.6	<0.001	R	0.091
8-iso-PGF2α (severe cases)	2 (3)	8.48	4.93, 12.03	<0.001	93.2	<0.001	R	0.044 (0.03)
TAC (all cases)	13 (18)	–1.15	–2.01, –0.30	0.008	97.3	<0.001	R	0.006 (0.008)
TAC (mild-moderate cases)	3 (4)	–5.79	–12.09, 0.51	0.072	98.9	<0.001	R	0.014 (0.072)
TAC (severe cases)	3 (3)	–2.80	–9.39, 3.79	0.405	99.1	<0.001	R	0.242
HEL	5 (5)	0.37	0.13, 0.62	0.003	24.3	0.259	F	0.066
OSI (all cases)	4 (6)	6.76	4.20, 9.31	<0.001	98.7	<0.001	R	0.025 (0.450)
OSI (mild-moderate cases)	2 (3)	14.00	5.39, 22.61	0.001	97.3	<0.001	R	0.005 (0.074)
OSI (severe cases)	2 (2)	5.76	4.36, 7.17	<0.001	69.6	0.070	R	-
Total thiol	4 (4)	–1.65	–3.24, –0.06	0.042	97.1	<0.001	R	0.297
3-NT	8 (29)	0.86	0.21, 1.51	0.010	93.0	<0.001	R	0.233
DT	4 (6)	0.66	0.52, 0.80	<0.001	31.4	0.200	F	0.004 (<0.01)
NFK	3 (5)	–0.19	–0.77, 0.39	0.525	89.7	<0.001	R	0.459

ASD, Autism spectrum disorder; TL, telomere length; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HEL, hexanolyl-lysine; TAC, total antioxidant capacity; 8-iso-PGF2α, 8-isoprostane; 3-NT, 3-nitrotyrosine; DT, dityrosine; NFK, N-formylkynurenine; OSI, oxidative stress index; SMD, standardized mean difference; CI, confidence interval; F, fixed-effects; R, random-effects; p_H-value, significance for heterogeneity; p_E-value, significance for effects; No., the number of articles (the number of experimental datasets).

was about 3:1 among ASD children [64]. First impressions have suggested males with ASD were rated lower than females on social cognition and object relations, emotional investment, and social causality scales [65,66]. Females decreased more in symptom severity than males across childhood [67]. These findings indicated that TL may be particularly decreased in male ASD patients, which was confirmed by this study. The negative associations of TL with female ASD may be resulted from the small sample size of them (relative to males) and needs to be further confirmed through increased statistical power.

The underlying mechanism of telomere shortening in ASD patients remains not completely understood, but activation of oxidative stress was considered as a major factor because Zhang *et al*. [10] found that decreased TL was positively correlated with lower activity of catalase (CAT) which is an important antioxidant enzyme for catalyzing H_2O_2 to H_2O and O_2 in maintaining the redox balance. The accumulated H_2O_2 due to lower activity of CAT may specifically attack the 8th carbon atom of the DNA guanine base in the telomere sequence and lead to the production of 8-OHdG [17,18,68]. The presence of oxidative lesions in the DNA can promote DNA single- or double-strand breaks at telomeric regions and cause the loss of the distal fragments of telomeric DNA and consequent telomere attrition [69,70]. To confirm oxidative DNA damage status in children and adolescents with ASD, studies with 8-OHdG in ASD patients were also retrieved. As expected, meta-analysis of 11 studies showed that subjects with ASD (particularly mild-moderate severity) had an increased 8-OHdG concentration, which was concordant with meta-analyses for mental illnesses [71,72].

Although multivariate analysis by Zhang *et al*. [10] found that CAT was an independent biomarker for prediction of ASD, meta-analysis of all evidence did not detect their significant association [16], indicating the necessity of identification of more effective oxidative stress biomarkers for young subjects with ASD. In this study, studies providing the data of 8-iso-PGF2α, OSI, TAC, HEL, thiol, 3-NT, DT and NFK in ASD patients and controls were incorporated. Meta-analysis showed that 8-iso-PGF2α, OSI, HEL, 3-NT and DT were significantly increased, whereas TAC was significantly decreased in ASD patients compared with controls. However, publication bias correction analysis excluded the significance of 8-iso-PGF2α and OSI. TAC is an assessment of the cumulative action of all antioxidants (including redox synergistic interactions) and may represent a better biomarker than individual anti-oxidants (such as CAT) [73]. Moreover, subgroup analysis showed that TAC in urine and saliva samples of ASD patients was significantly changed, but not in blood, indicating urine is more valuable biological matrix for TAC assay [35].

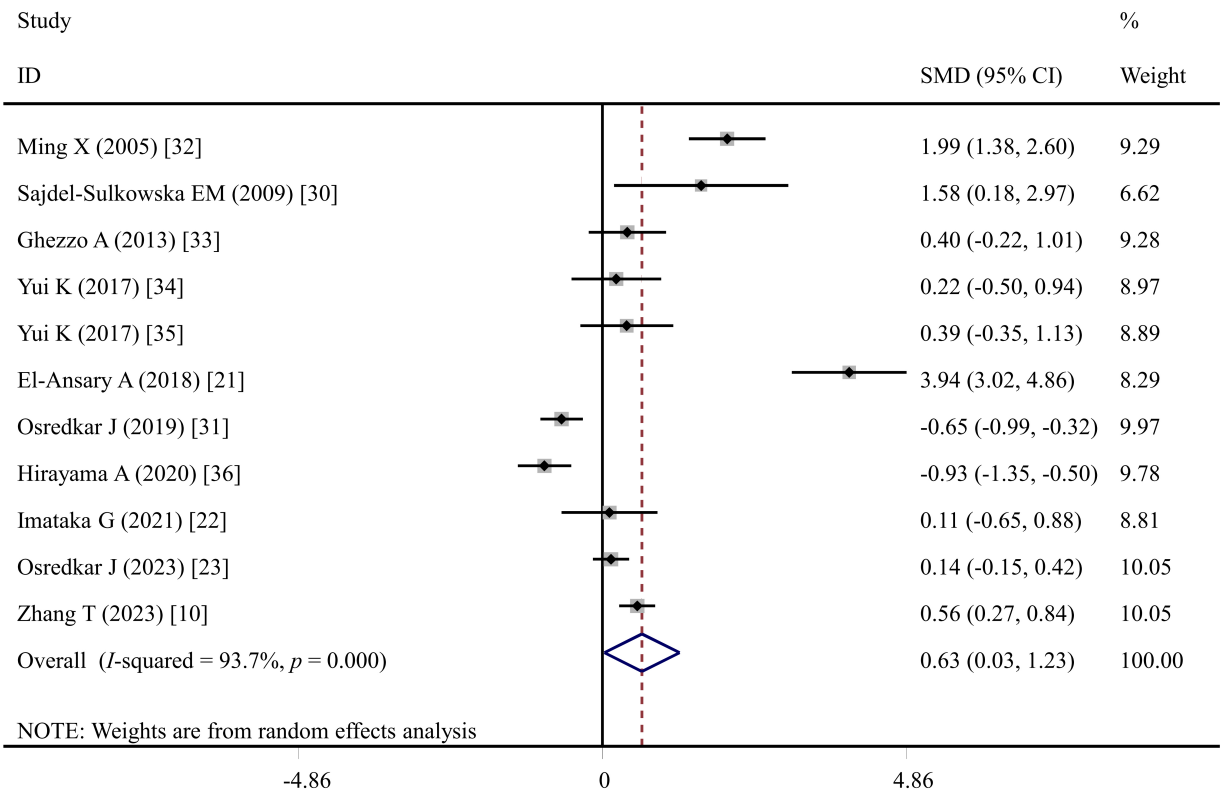

Fig. 3. Forest plots to show the effect size that compared the 8-OHdG in autism patients with that in the control group. SMD, standardized mean difference; CI, confidence interval.

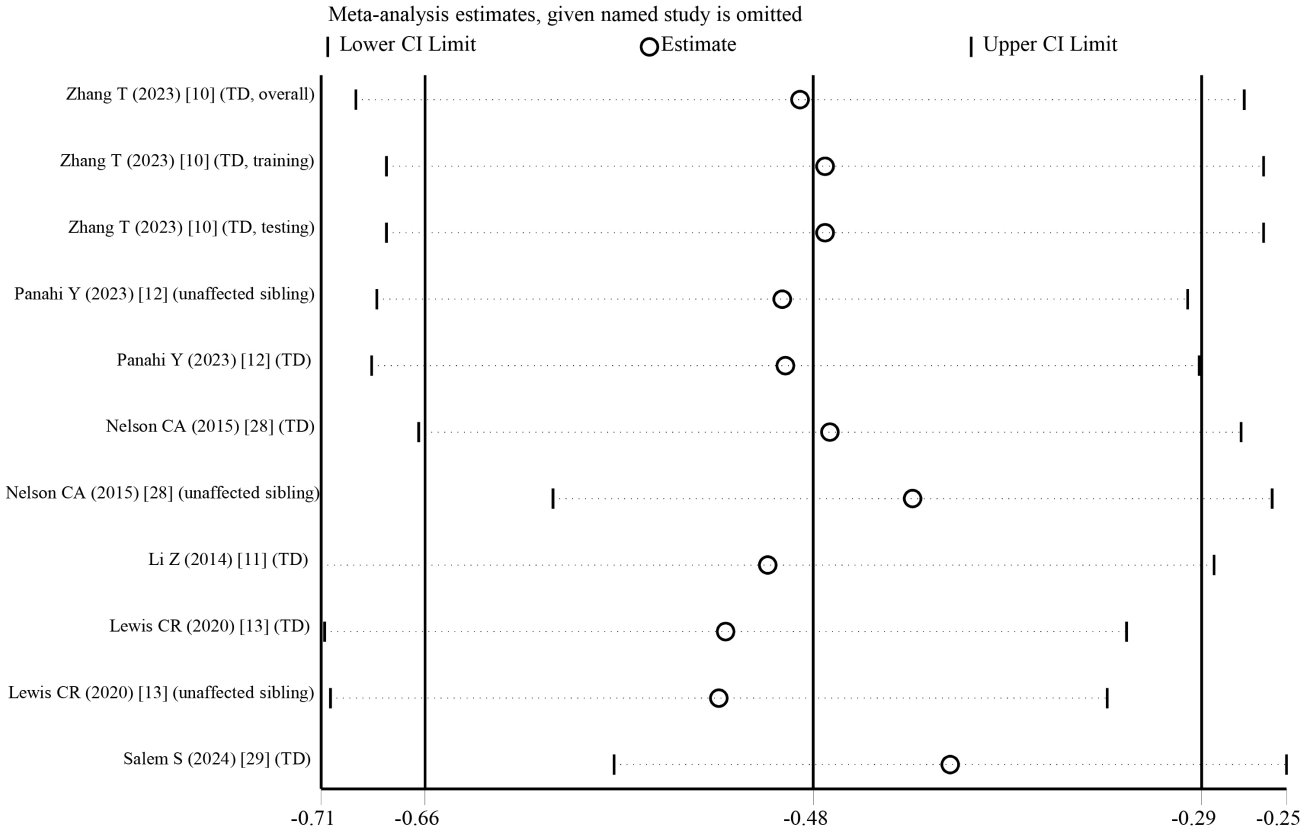

Fig. 4. Sensitivity analysis for the telomere length. CI, confidence interval.

With the exception of DNA, oxidative stress also causes lipid and protein damages. HEL is a lipid peroxidation biomarker formed by the reaction of lysine with peroxidized n-6 polyunsaturated fatty acid (PUFA) [74]. n-6 PUFA has been reported to exert protective roles against neuronal degeneration and neuronal loss in experimental rats with valproic acid-induced autism [75]. A decline in levels of n-6 PUFA to cause HEL elevation due to oxidative stress has been found as a risk factor for inducing autism [22,35,76]. 3-NT is a marker of tyrosine nitration of proteins, which is generated through a reaction between the tyrosyl radical and nitrogen dioxide [77], whereas DT is a marker of oxidation in the tyrosine residue of proteins, which is derived from a reaction between the tyrosyl radical and reactive oxygen species. An *in vivo* experiment has demonstrated that compared to free-tyrosine, free-3-NT (nitration of amino acids) treatment induces more nigrostriatal cell body loss and increases net ipsilateral turning behavior (consistent with degeneration of striatal dopaminergic nerve terminals) [78]. An *in vitro* study has also proved that introduction of 3-NT can damage the cytoskeleton and induce apoptosis of neuronal PC12 cells [79]. Compared with the saline group, DT administration has been observed to impair hippocampus-dependent nonspatial memory in young adulthood mice [80]. Therefore, decreased TAC and increased HEL, 3-NT and DT may be potential biomarkers reflecting the oxidative stress status of ASD.

Several limitations should be acknowledged when interpreting results reported here. First, the number of published studies was relatively small (<10 for most indicators except of TAC and 8-OHdG) and the sample size in these studies was not large (<100 for most articles), which may lead to underestimation or overestimation of the effect size for variables. Additionally, Egger's linear regression test showed the presence of publication bias for 8-iso-PGF2α and OSI and the trim-and-fill correction analysis removed the significance of them in ASD, indicating "missing" studies obviously influenced the results. Subgroup analysis for TL detected the heterogeneity was removed when the sample size was ≥ 100. Meta-regression analysis also confirmed the sample size as a moderator variable on effect sizes of TL. Second, Qasem *et al.* [37] found that age had a significant negative correlation with 8-iso-PGF2α plasma levels in ASD patients. Hirayama *et al.* [36] reported that 8-OHdG was only significantly increased in ASD children with an age under 6 years compared to that in the age-matched male TD group, not significantly changed in the analysis of all cases. TL of ASD patients was identified to be progressively reduced with an increase in the chronological age [12,81]. These findings indicate biomarkers may be useful as a screening test for specific age or sex group. However, no raw data for each patient with different behavioral and clinicopathological characteristics (e.g., age, sex) were available and thus subgroup or meta-regression analyses based on these risk factors could not be performed for all indicators. Third, the receiver operating characteristic curve was rarely drawn to confirm the diagnostic efficiency of TL [10] and oxidative stress biomarkers [49]. Detection method for biomarkers in different articles also varied. Therefore, which length or concentration should be designed as cut-off values for diagnosis of ASD remains uncertain. Fourth, this meta-analysis evaluated the associations between ASD and biomarkers based on the univariate analysis results in included articles. Multivariate analysis data were rarely available [10,11]. Fifth, accumulation of oxidative damage at telomeres and telomere shortening also suggested to be attributed to decreased DNA repair [82,83]. Recovering DNA repair gene expression can ameliorate autism-like behaviors in mice [84]. However, until now, no human studies detected the expression of DNA repair genes. Sixth, there was a lack of sufficient studies that analyzed the changes of selected biomarkers in adult ASD subjects, which could be interesting for future longitudinal study of ASD. Accordingly, future analyses in larger, more diverse populations with detailed individual information are warranted to powerfully confirm the connection of TL, oxidative stress biomarkers and DNA repair genes with ASD before clinical use.

5. Conclusions

This meta-analysis demonstrates that children and adolescents with ASD are associated with significantly shorter TL, lower TAC and higher oxidative DNA (8-OHdG), lipid (HEL) and protein (3-NT and DT) damages. These indicators may have the potential to be used as biomarkers for early prediction of ASD in children and adolescents. However, more clinical researches in larger, more diverse ASD populations are still needed in order to provide a strong evidence for these biomarkers because the number of articles for most indicators is relatively less.

Abbreviations

ASD, Autism spectrum disorder; TL, telomere length; TD, typical development; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-iso-PGF2α, 8-isoprostane; HEL, hexanolyl-lysine; TAC, total antioxidant capacity; 3-NT, 3-nitrotyrosine; DT, dityrosine; NFK, N-formylkynurenine; OSI, oxidative stress index; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay methods; LC-MS/MS, liquid chromatography-tandem mass spectrometry; GC-MS, gas chromatography–mass spectrometry; ORAC, oxygen radical absorbance capacity; FRAP, ferric-reducing antioxidant power; ABTS, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) cation bleaching; PM, phosphomolybdate; NOS, Newcastle–Ottawa Scale; SMD, standardized mean difference; CI, confidence interval; T/S, telomere to single-copy gene sequence ratio; CAT, catalase; PUFA, polyunsaturated fatty acid.

Availability of Data and Materials

The data used for meta-analysis in the study were included in the included articles and **Supplementary Tables**.

Author Contributions

LM: Conceptualization, Data curation, Formal analysis, Writing—original draft. CL: Data curation, Investigation. RS: Methodology, Validation. YQ: Methodology, Writing— review & editing. FZ: Conceptualization, Supervision, Writing—review & editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/JIN24948.

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