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A meta-analysis of pro-inflammatory cytokines in autism spectrum disorders: Effects of age, gender, and latitude

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ABSTRACT

Background: Autism spectrum disorders (ASD) occur in 1.5% of the general population worldwide. Studies suggest that ASD might have more costs than diabetes and attention deficit and hyperactivity disorder by 2025. Dysregulation of the cytokine system is well-documented in ASD. We conducted a meta-analysis of studies providing data on circulating concentrations of pro-inflammatory cytokines in people with ASD compared with control subjects without ASD.

Methods: We identified potentially eligible studies by systematically searching electronic databases from inception to February 2018.

Results: Thirty-eight studies with total of 2487 participants (1393 patients with ASD and 1094 control subjects) were included in the meta-analysis; 13 for interferon (IFN)-γ, 17 for interleukin (IL)-1β, 22 for IL-6, 19 for tumor necrosis factor (TNF)-α, 4 for IL-1α, 6 for IL-2, 4 for IL-7, 8 for IL-8, 14 for IL-12, 3 for IL-15, 12 for IL-17, 3 for IL-18, 3 for IL-2 receptor, 3 for TNF-β, and 3 for IL-23. We found medium increases in levels of plasma IFN-γ (standardized mean difference, SMD = 0.53) and serum IL-1β (SMD = 0.56) and small increases in levels of blood IL-1β (SMD = 0.35), serum IL-6 (SMD = 0.30) and serum TNF-α (SMD = 0.31) for patients with ASD. Meta-regression analyses identified latitude as a negative moderator of the effect size (ES) of difference in mean levels of IFN-γ (R^2 = 0.26) and TNF-α (R^2 = 0.74). Also, difference in the mean age between patients and controls had a negative interaction with the ES of difference in mean levels of IL-1β. In contrast, there was a positive effect of the moderator of difference in the proportion of male subjects between patients and controls on the ES of difference in mean levels of IL-1β. We found no significant alterations in peripheral levels of other pro-inflammatory cytokines including IL-1α, IL-2, IL-2R, IL-3, IL-7, IL-8, IL-12, IL-12p40, IL-12p70, IL-15, IL-17, IL-18, IL-23, TBF-β, and TNFRI/II in patients with ASD.

Conclusions: This meta-analysis provides evidence for higher concentration of pro-inflammatory cytokines IFN-γ, IL-1β, IL-6, and TNF-α in autistic patents compared with control subjects. Also, meta-regression analyses point to the interaction of latitude, age, and gender with peripheral alterations of associated pro-inflammatory cytokines.

1. Introduction

Autism spectrum disorders (ASD) affecting 1.5% of the population worldwide are projected to be increasing in the upcoming decade and might have more costs than diabetes and attention deficit and hyperactivity disorder by 2025 (Leigh and Du, 2015). Forty years of endeavours remark ASD as the result of complex interactions between varieties of origins especially genetics, epigenetics, and the environment. However, there is yet no definite medical plan for diagnosis and the future care of our children with ASD. A basic element of such planning lies in understanding the pathophysiological mechanisms that underpin ASD, among which is immune dysfunction. The origin of the

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link between autism and immunity goes back to 1970s when reports of ASD being detected in children with a history of maternal infection during pregnancy or after exposure to measles, mumps, and rubella vaccination appeared. ASD is now considered a brain condition where both the innate and adaptive immunity are impaired (Jyonouchi et al., 2002; Jyonouchi et al., 2001). In line with this are the frequent incidents of immune disorders – ranging from atopy, food allergy, and viral infections to asthma, primary immunodeficiency, and autoimmune disorders – in patients with ASD and their parents (Bakkaloglu et al., 2008; Libbey et al., 2005; Lucarelli et al., 1995). Evidence of neuroglial activation and focal brain inflammation in subjects with ASD implies that the CNS immunity is influenced by ASD as well (Li et al., 2009; Pardo et al., 2005; Vargas et al., 2005).

Research suggests dysregulation of the cytokine system as the core of immunopathogenesis of ASD. Analyses of brain tissues indicate the increase of proinflammatory chemokines in patients with ASD (Vargas et al., 2005). Also, in vitro studies provide evidence of augmented production of proinflammatory and regulatory cytokines by peripheral blood mononuclear cells (PBMCs) in patients with ASD (Jyonouchi et al., 2001; Molloy et al., 2006). Children with ASD are more likely to have gastrointestinal symptoms than typically developed children (Valicenti-McDermott et al., 2006). Supporting this, studies have demonstrated that spontaneous production of proinflammatory cytokines by mucosal lymphocytes (Ashwood et al., 2004) as well as production of cytokines against common dietary proteins (Jyonouchi et al., 2005a) is increased in children with ASD. Interestingly, inflammatory responses have been shown to correlate with severity of behavioral symptoms in ASD (Ashwood et al., 2011a; Ashwood et al., 2011c).

Studies using convenient biomaterials e.g. whole blood, plasma, and serum have frequently investigated whether patients with ASD exhibit alteration of cytokine profile compared to controls without ASD. The associated findings are not in the same direction (Al-Ayadhi and Mostafa, 2012; Ashaat et al., 2017; Ashwood et al., 2008; Ashwood et al., 2011b; Barbosa et al., 2015; Bryn et al., 2017; Businaro et al., 2016; Croonenberghs et al., 2002; Denney et al., 1996; El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2016; El-Ansary et al., 2011; El Gohary et al., 2015; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Enstrom et al., 2008; Ghaffari et al., 2016; Guloksuz et al., 2017; Han et al., 2017; Hashim et al., 2013; Ibrahim et al., 2015; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Okada et al., 2007; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Saresella et al., 2016; Shaker et al., 2016; Singh, 1996; Singh et al., 1991; Suzuki et al., 2011; Sweeten et al., 2004; Tobiasova et al., 2011; Tonhajzerova et al., 2015; Tostes et al., 2012; Tsilioni et al., 2015; Xie et al., 2017; Yang et al., 2015a; Yang et al., 2015b; Zimmerman et al., 2005). A systematic review and meta-analysis by Masi et al. (2015) helped to reduce this heterogeneity (Masi et al., 2015). The number of included studies (n = 17) was, however, small at that time. Numerous studies examining concentrations of pro-inflammatory cytokines in patients with ASD have been published thereafter (Ashaat et al., 2017; Barbosa et al., 2015; Bryn et al., 2017; Businaro et al., 2016; El-Ansary et al., 2016; El Gohary et al., 2015; Ghaffari et al., 2016; Guloksuz et al., 2017; Han et al., 2017; Ibrahim et al., 2015; Jacome et al., 2016; Pardo et al., 2017; Pecorelli et al., 2016; Saresella et al., 2016; Shaker et al., 2016; Tonhajzerova et al., 2015; Tsilioni et al., 2015; Xie et al., 2017; Yang et al., 2015a; Yang et al., 2015b). The present meta-analysis was designed to provide an update meta-analysis of studies that have determined cytokine measurements in patients with ASD compared to controls without ASD.

2. Materials and methods

We prepared the present systematic review and meta-analysis study in the same manner to our previously reported meta-analytic studies (Agah et al., 2018; Harsini et al., 2018; Rezaei et al., 2017; Saghazadeh et al., 2017a; Saghazadeh et al., 2014; Saghazadeh et al., 2017b; Saghazadeh et al., 2015; Saghazadeh and Rezaei, 2017a, b) and based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Moher et al., 2009). The PRISMA statement is a 27-item checklist, which has a rational design for improving the quality of reporting systematic review and meta-analysis studies. Before the study is conducted, the authors (A.S., A.H., and N.R.) developed study protocol that is available on request.

2.1. Search strategy

We identified potentially eligible studies by systematically searching the databases PubMed (1967–February 2018), Scopus (1965–February 2018), and Web of Science (1991–February 2018). The search strategy was developed using the combination of following keywords: (human OR subject OR participant OR volunteer OR patient OR people OR person OR case OR control OR individual OR population OR case-control OR child OR children OR kid OR adolescent OR adult) AND (autism OR autistic OR Asperger OR Asperger's OR pervasive developmental disorder OR pervasive developmental delay) AND (cytokine OR interleukin OR interferon OR Interleukin-1 OR IL-1 OR IL-1beta OR IL-1B OR IL-1alpha OR IL-1A OR IL1RN OR IL1RA OR IL-1RA OR IL-1R1 OR Interleukin-2 OR IL-2 OR Interleukin-3 OR IL-3 OR Interleukin-4 OR IL-4 OR Interleukin-5 OR IL-5 OR Interleukin-6 OR IL-6 OR Interleukin-7 OR IL-7 OR Interleukin-8 OR IL-8 OR Interleukin-9 OR IL-9 OR Interleukin-10 OR IL-10 OR Interleukin-11 OR IL-11 OR Interleukin-12 OR IL-12 OR IL12A OR IL12B OR Interleukin-13 OR IL-13 OR Interleukin-15 OR IL-15 OR Interleukin-16 OR IL-16 OR Interleukin-17 OR IL-17 OR IL-17A OR IL-17F OR Interleukin-18 OR IL-18 OR IL18 OR Interleukin-20 OR IL-20 OR Interleukin-21 OR IL-21 OR Interleukin-22 OR IL-22 OR Interleukin-23 OR IL-23 OR IL-23A OR IL23A OR Interleukin-24 OR IL-24 OR Interleukin-27 OR IL-27 OR transforming growth factor beta OR TGF OR tgf-beta OR tgf-b OR TGF-b1 OR TGF-b2 OR TGF-beta1 OR TGF-beta2 OR tgf-beta OR TGF-beta1 OR TGF-beta2 OR interferon gamma OR ifn-gamma OR OR ifng OR IFNA1 OR IFNA2 OR ifn-alpha OR IFN-alpha1 OR IFNalpha1 OR IFN-alpha2 OR tumor necrosis factor OR TNF OR tnf-alpha OR tnfa OR tnf-beta OR tnf beta OR tnfb OR TNFRSF11B OR TNFSF10 OR TNFSF11 OR TNFSF13B). See supplemental information for specific search strategies designed for each of the main electronic medical databases. The database search was not restricted to any time period, language, or any location. To better control publication bias, we searched Google Scholar for additional articles that may have been missed on the initial search. Database searches were supplemented with a manual search of reference lists from the identified articles and relevant reviews. The last search was conducted in February 2018.

2.2. Selection criteria

Studies providing information on concentrations of pro-inflammatory cytokines in blood, plasma, or serum among patients with ASD were considered for review. The inclusion criteria were as follows: (1) subjects with ASD and (2) cross-sectional or follow-up studies comparing concentrations of pro-inflammatory cytokines in ASD patients with healthy control subjects. Exclusion criteria were: (1) studies that included ASD patients with specific features e.g. chromosomal abnormalities, (2) studies that measured cytokines other than of interest e.g. gamma-interferon-inducible protein, chemokines, and osteopontin, (3) studies that employed sources other than of interest e.g. peripheral blood mononuclear cells (PBMC), monocytes, intestinal biopsies, neonatal blood spot (NBS), and lymphoblasts for measurement of cytokines, (4) lack of a control group, (5) duplicate records, (6) lack of enough data for meta-analysis, and (7) sorts of publications other than original articles e.g. Letter and correspondence. The authors (BA, KK, and AA) independently made the decision to include or not include a particular study after a detailed review with the above criteria, and if there was any disagreement, the first author (AS) was consulted.

2.3. Data extraction

Four authors (AS, BA, KK, and AA) independently extracted the following data from each included publication; first-named author, year of publication, location, the cytokine that was measured, the assay, source (serum, plasma, or whole blood), and scale that were employed for cytokine measurement, number of subjects, demographic characteristics (e.g. age and sex), and cytokine levels (mean and SD) in both the patient and the control groups. Discrepancies in any item was resolved after discussion and consensus was reached. When included publications did not contain enough information for meta-analysis, we contacted the corresponding authors and requested for additional data. If authors could not provide us necessary data, then we a. used digital ruler if the data were presented in graphical figures and b. transformation formula if the data were presented in other formats e.g. median, standard error, and interquartile range (IQR).

2.4. Quality assessment

As recommended by the Cochrane Collaboration (Higgins JPT), the quality of studies included in the between-group meta-analyses was assessed using the Newcastle–Ottawa Scale (NOS) designed for case-control studies(Stang, 2010). It is composed for the assessment of three main aspects of case-control studies; sample selection, comparability of cases and controls, and exposure with a maximum of 8 stars. The total quality score is simply calculated as the sum of the frequency of criteria that were met by the particular study. As shown in Supplementary Table 1, the quality score of the included studies varied from 4 to 8 with mean score of 5.6 (SD = 1.0).

2.5. Statistical analysis

All between-group meta-analyses were performed, using Review Manager (version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014). We created the continuous type of outcome and entered the number of participants in experimental and control groups and mean and SD of cytokine levels. Because studies used different measurement scales or assays, we employed the standardized mean difference (SMD) for measurement of effect. The term effect size (ES) generally refers to the difference between the two groups. In particular, the SMD and its associated 95% confidence interval (CI) were used to estimate differences in cytokine levels between ASD patients and HC. As explained in (Higgins and Green, 2011), the ES of 0.2, 0.5, and 0.8 represent small, moderate, and large effect estimates, respectively.

We explored heterogeneity across studies using the Cochran's Q test that is computed by the weighted sum of squared differences between individual study ES estimates. A P-value of 0.10 or less indicates the presence of heterogeneity. The I^2 index was also applied to have a more precisely quantitative estimate of heterogeneity. According to the Cochrane guidelines, the I^2 less than 40% would mean that the heterogeneity across studies might be not important. In this case, the fixed-effects model is preferred for meta-analysis. When the I^2 estimates fluctuated more than 40%, the random-effects approach was chosen as the meta-analysis model.

We also employed comprehensive meta-analysis Software version 3.0 (Borenstein, NH, USA) in meta-regression analyses. Univariate meta-regression analyses were conducted to investigate effect of potential moderators e.g. latitude, difference in the mean age of patients and controls (years), difference in the percentage of male subjects between patients and controls, publication year, and sample size on the effect sizes.

Publication bias was assessed using the degree of funnel plot asymmetry and with the Begg-Mazumdar Kendall's tau (Begg and Mazumdar, 1994) and Egger bias test (Duval and Tweedie, 2000). In fact, funnel plots are primarily used to visually detect publication bias. While the Begg-Mazumdar Kendall's tau and Egger bias test are objective measures that help users confirm visual cues provided by funnel plots. The trim-and-fill method was used to adjust effect sizes for which there was evidence of publication bias (Duval and Tweedie, 2000).

3. Results

3.1. Study selection

The database search resulted in 1770 records. After removal of duplicates (n = 844), the title/abstract of 926 discrete search results was screened for potential eligibility. 852 papers were excluded through screening. 74 full-text articles were reviewed in detail and 42 met the inclusion criteria. Of these, 38 studies with a total of 2487 participants (1393 patients with ASD and 1094 controls) were included in the mate-analysis of pro-inflammatory cytokines in ASD (Al-Ayadhi and Mostafa, 2012; Ashaat et al., 2017; Ashwood et al., 2011b; Barbosa et al., 2015; Bryn et al., 2017; Businaro et al., 2016; Croonenberghs et al., 2002; Denney et al., 1996; El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2016; El-Ansary et al., 2011; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Enstrom et al., 2008; Ghaffari et al., 2016; Guloksuz et al., 2017; Hashim et al., 2013; Ibrahim et al., 2015; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Saresella et al., 2016; Shaker et al., 2016; Singh, 1996; Singh et al., 1991; Suzuki et al., 2011; Sweeten et al., 2004; Tobiasova et al., 2011; Tonhajzerova et al., 2015; Tostes et al., 2012; Tsilioni et al., 2015; Xie et al., 2017; Yang et al., 2015a; Yang et al., 2015b; Zimmerman et al., 2005). With enough data (at least three between-group comparisons for each cytokine), meta-analysis was conducted for data on 19 pro-inflammatory cytokines (IFN-γ, IL-1α, IL-1β, IL-2, IL-2R, IL-3, IL-6, IL-7, IL-8, IL-12, IL-12p40, IL-12p70, IL-15, IL-17, IL-18, IL-23, TNF-α, TBF-β, and TNFRI/II) in ASD. Fig. 1 provides an overview of study selection for systematic review and meta-analysis as recommended by PRISMA guidelines.

3.2. Study and patient characteristics

Studies were published between 1991 and 2017. Ten studies were conducted in the United States of America (Ashwood et al., 2011b; Denney et al., 1996; Enstrom et al., 2008; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Singh, 1996; Singh et al., 1991; Sweeten et al., 2004; Tobiasova et al., 2011; Tsilioni et al., 2015; Zimmerman et al., 2005), six in Italy (Businaro et al., 2016; Croonenberghs et al., 2002; Emanuele et al., 2010; Pecorelli et al., 2016; Ricci et al., 2013; Saresella et al., 2016), six in Egypt (Al-Ayadhi and Mostafa, 2012; Ashaat et al., 2017; El Wakkad and Saleh, 2006; Hashim et al., 2013; Ibrahim et al., 2015; Shaker et al., 2016), three in China (Xie et al., 2017; Yang et al., 2015a, 2015b), three in Saudi Arabia (El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2011, 2016), two in Brazil (Barbosa et al., 2015; Tostes et al., 2012), and one study in each of Cuba (Jacome et al., 2016), Iran (Ghaffari et al., 2016), Slovakia (Tonhajzerova et al., 2015), Turkey (Guloksuz et al., 2017), Japan (Suzuki et al., 2011), and Norway (Bryn et al., 2017). Plasma was used for cytokine measurements in 19 studies (Ashwood et al., 2011b; Barbosa et al., 2015; Denney et al., 1996; El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2016; El-Ansary et al., 2011; Enstrom et al., 2008; Guloksuz et al., 2017; Hashim et al., 2013; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Singh, 1996; Suzuki et al., 2011; Sweeten et al., 2004; Tonhajzerova et al., 2015; Tostes et al., 2012; Yang et al., 2015a; Yang et al., 2015b), serum in 18 studies (Al-Ayadhi and Mostafa, 2012; Bryn et al., 2017; Businaro et al., 2016; Croonenberghs et al., 2002; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Ghaffari et al., 2016; Han et al., 2017; Ibrahim et al., 2015; Okada et al., 2007; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Shaker et al., 2016; Tobiasova et al., 2011; Tsilioni et al., 2015; Xie et al., 2017; Zimmerman et al., 2005), and both plasma and serum

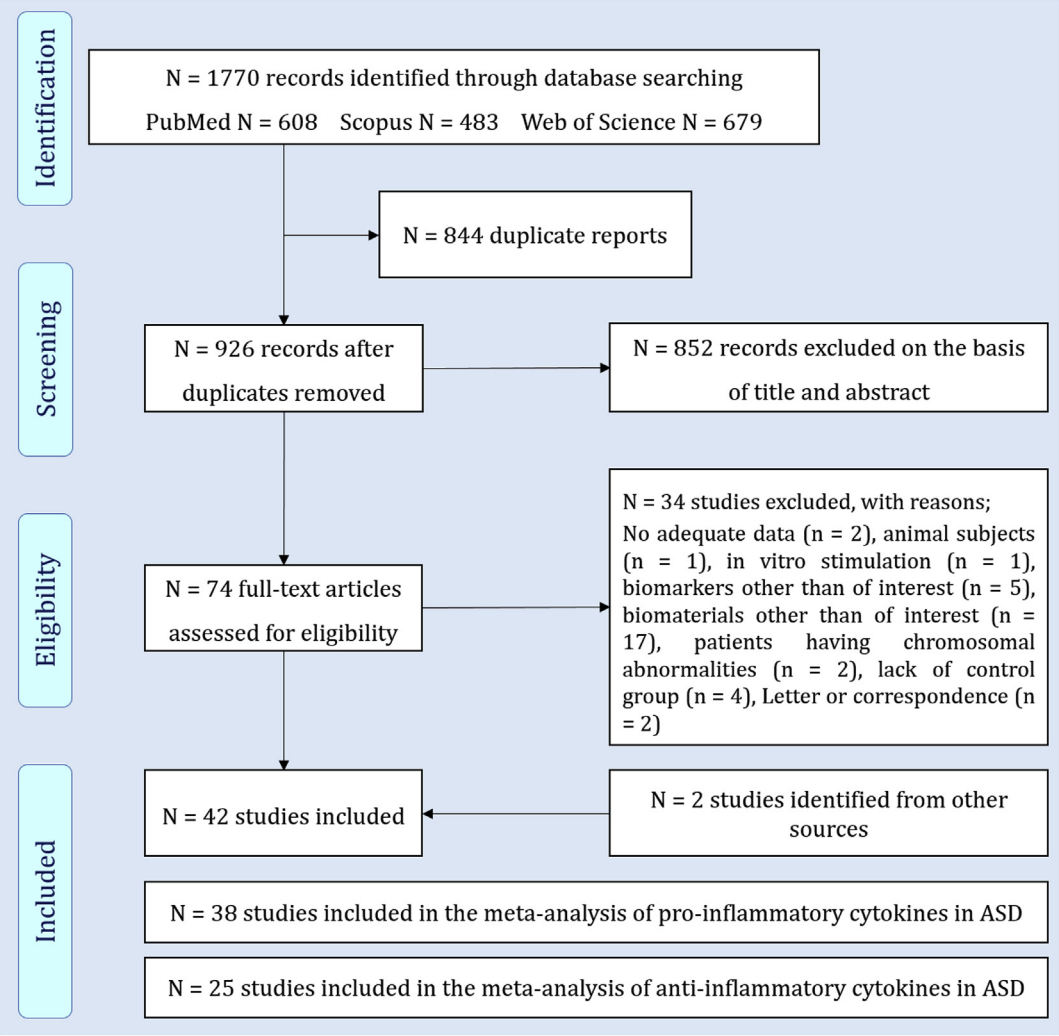

Fig. 1. PRISMA flowchart of study selection for systematic review and meta-analysis of pro-inflammatory cytokines in ASD.

in one study (Singh et al., 1991). The enzyme-linked immunosorbent assay (ELISA) was used as the analytical procedure for detection of cytokines in most of the included studies (n = 27) (Al-Ayadhi and Mostafa, 2012; Ashaat et al., 2017; Barbosa et al., 2015; Businaro et al., 2016; Croonenberghs et al., 2002; Denney et al., 1996; El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2016; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Enstrom et al., 2008; Ghaffari et al., 2016; Hashim et al., 2013; Ibrahim et al., 2015; Jacome et al., 2016; Napolioni et al., 2013; Ricci et al., 2013; Saresella et al., 2016; Shaker et al., 2016; Singh, 1996; Singh et al., 1991; Sweeten et al., 2004; Tonhajzerova et al., 2015; Tsilioni et al., 2015; Yang et al., 2015a; Yang et al., 2015b; Zimmerman et al., 2005). Multiplex techniques were used in seven studies (Ashwood et al., 2011b; Bryn et al., 2017; Manzardo et al., 2012; Pardo et al., 2017; Suzuki et al., 2011; Tobiasova et al., 2011; Xie et al., 2017), both multiplex techniques and ELISA in two studies (Guloksuz et al., 2017; Pecorelli et al., 2016), and both flow cytometry and ELISA in one study (Tostes et al., 2012). One study lacked information in this context (El-Ansary et al., 2011). Supplementary Tables 1 and 2 summarize characteristics and quality of the included studies.

Patients with ASD and controls without ASD were matched for age and sex in 34 studies (89.5%) (Al-Ayadhi and Mostafa, 2012; Ashaat et al., 2017; Ashwood et al., 2011b; Barbosa et al., 2015; Businaro et al., 2016; Croonenberghs et al., 2002; Denney et al., 1996; El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2016; El-Ansary et al., 2011; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Enstrom et al., 2008; Ghaffari et al., 2016; Guloksuz et al., 2017; Hashim et al., 2013; Ibrahim et al., 2015; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Ricci et al., 2013; Saresella et al., 2016; Shaker et al., 2016; Singh, 1996; Singh et al., 1991; Suzuki et al., 2011; Sweeten et al., 2004; Tonhajzerova et al., 2015; Tostes et al., 2012; Xie et al., 2017; Yang et al., 2015a; Yang et al., 2015b; Zimmerman et al., 2005). There were studies that included control subjects with similar values for body mass index (Guloksuz et al., 2017; Yang et al., 2015a, 2015b), race (Croonenberghs et al., 2002; Denney et al., 1996; Sweeten et al., 2004), ethnicity (Napolioni et al., 2013; Suzuki et al., 2011), and economic class (Denney et al., 1996) as well. Patients and controls were matched only for age (Bryn et al., 2017; Pecorelli et al., 2016; Tsilioni et al., 2015) or sex (Tobiasova et al., 2011) in four studies. Information regarding the number of male and female subjects was available in most of the included studies (Al-Ayadhi and Mostafa, 2012; Ashaat et al., 2017; Ashwood et al., 2011b; Barbosa et al., 2015; Bryn et al., 2017; Croonenberghs et al., 2002; Denney et al., 1996; El-Ansary and Al-Ayadhi, 2012; El-Ansary et al., 2011; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Enstrom et al., 2008; Ghaffari et al., 2016; Guloksuz et al., 2017; Hashim et al., 2013; Ibrahim et al., 2015; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Ricci et al., 2013; Saresella et al., 2016; Shaker et al., 2016; Singh, 1996; Singh et al., 1991; Suzuki et al., 2011; Sweeten et al., 2004; Tobiasova et al., 2011; Tonhajzerova

Table 1
Statistics on meta-analyses regarding blood concentrations of pro-inflammatory cytokines in ASD.

Meta-analyses of blood pro-inflammatory cytokines in ASD														
Cytokine	Between-group ASD vs HC	No. of Pairwise	No. of subjects		Meta-analysis				Heterogeneity			Publication bias	
			Case	Control	SMD	95% CI		P-value	I^2%	Chi^2	P-value	P value of Begg's test	P value of Egger's test
IFN-γ	All	16	668	446	0.26	−0.05	0.57	0.10	82	83.12	< 0.00001	0.034	0.016
IFN-γ	Plasma	**10**	**382**	**305**	**0.53**	**0.05**	**1.00**	**0.03**	**88**	**73.31**	**< 0.00001**	**0.049**	**0.020**
IFN-γ	Serum	6	286	141	−0.11	−0.32	0.09	0.28	0	3.44	0.63	0.707	0.815
IL-12	All	4	145	112	1.07	−0.26	2.41	0.11	95	63.82	< 0.00001	0.308	0.221
IL-12p40	All	10	451	301	0.27	−0.08	0.61	0.13	78	41.85	< 0.00001	0.592	0.427
IL-12p40	Plasma	6	266	195	0.20	−0.18	0.57	0.31	70	16.63	0.005	0.707	0.843
IL-12p40	Serum	4	185	106	0.41	−0.33	0.16	0.28	88	25.06	< 0.0001	0.089	0.038
IL-12p70	All	11	427	277	0.26	−0.32	0.84	0.38	92	121.15	< 0.00001	0.876	0.809
IL-12p70	Plasma	5	169	108	0.41	−0.28	1.11	0.24	85	27.43	< 0.0001	1.000	0.797
IL-12p70	Serum	6	258	169	0.12	−0.82	1.05	0.81	95	93.17	< 0.00001	1.000	0.689
IL-15	All	7	301	159	0.03	−0.17	0.23	0.78	0	4.93	0.42	0.707	0.839
IL-15	Plasma	3	124	65	0.05	−0.25	0.36	0.73	43	3.51	0.17	1.000	0.582
IL-15	Serum	4	177	94	0.01	−0.25	0.27	0.94	0	1.38	0.50	0.296	0.121
IL-17	All	16	710	454	0.14	−0.24	0.51	0.48	89	131.75	< 0.00001	0.620	0.408
IL-17	Plasma	8	319	213	0.13	−0.50	0.76	0.68	91	76.52	< 0.00001	0.386	0.096
IL-17	Serum	8	391	241	0.16	−0.30	0.62	0.50	86	49.45	< 0.00001	0.711	0.683
IL-18	All	4	125	117	0.56	−1.01	2.14	0.48	96	73.53	< 0.00001	0.734	0.748
IL-18	Serum	3	97	89	0.74	−1.79	3.27	0.56	97	71.67	< 0.00001	1.000	0.849
IL-1α	All	8	394	198	0.04	−0.16	0.24	0.67	22	9.00	0.25	0.711	0.310
IL-1α	Plasma	4	152	93	−0.08	−0.48	0.33	0.70	55	6.63	0.08	0.734	0.611
IL-1α	Serum	4	242	105	0.13	−0.10	0.36	0.28	0	1.29	0.73	1.000	0.899
IL-1β	All	**20**	**704**	**548**	**0.35**	**0.08**	**0.61**	**0.01**	**79**	**92.49**	**< 0.00001**	**0.183**	**0.374**
IL-1β	Plasma	11	404	319	0.20	−0.01	0.42	0.06	45	18.33	0.05	0.640	0.476
IL-1β	Serum	**9**	**300**	**229**	**0.56**	**−0.00**	**1.13**	**0.05**	**89**	**71.79**	**< 0.00001**	**0.048**	**0.155**
IL-2	All	11	477	331	0.04	−0.14	0.22	0.63	31	14.40	0.16	0.755	0.679
IL-2	Plasma	5	245	176	0.04	−0.18	0.27	0.70	14	4.66	0.32	0.221	0.259
IL-2	Serum	6	232	155	0.07	−0.23	0.37	0.66	49	9.73	0.08	0.260	0.570
IL-23	All	3	139	99	−1.06	−3.16	1.05	0.33	98	89.25	< 0.00001	1.000	0.336
IL-2R	All	3	46	54	−0.11	−0.50	0.28	0.58	0	0.03	0.98	1.000	0.157
IL-3	All	6	292	154	−0.02	−0.22	0.19	0.87	0	3.89	0.87	0.707	0.864
IL-3	Plasma	3	127	68	−0.06	−0.39	0.28	0.73	0	0.13	0.72	1.000	0.436
IL-3	Serum	3	165	86	0.03	−0.33	0.40	0.85	45	3.67	0.16	0.296	0.255
IL-6	All	**25**	**857**	**622**	**0.33**	**0.01**	**0.66**	**0.04**	**48**	**23.19**	**0.03**	**0.528**	**0.602**
IL-6	Plasma	12	439	349	0.37	−0.26	1.00	0.25	94	174.25	0.25	0.945	0.756
IL-6	Serum	**13**	**418**	**273**	**0.30**	**0.07**	**0.52**	**0.01**	**48**	**23.19**	**0.03**	**0.669**	**0.536**
IL-7	All	8	329	187	−0.14	−0.44	0.15	0.34	57	16.43	0.02	0.902	0.818
IL-7	Plasma	4	152	93	−0.05	−0.53	0.43	0.83	68	9.24	0.03	0.734	0.875
IL-7	Serum	4	177	94	−0.24	−0.62	0.13	0.21	49	5.83	0.12	0.734	0.790
IL-8	All	9	362	253	0.25	−0.10	0.60	0.16	74	30.20	0.0002	0.466	0.250
IL-8	Plasma	6	264	200	0.25	−0.25	0.75	0.32	83	29.12	< 0.0001	0.260	0.400
IL-8	Serum	3	98	53	0.27	−0.07	0.60	0.12	0	0.78	0.68	1.000	0.463
sTNFRI/II	All	4	120	70	0.22	−0.09	0.53	0.17	0	2.82	0.42	0.308	0.322
TNF-α	All	22	823	587	0.21	−0.07	0.49	0.14	67	24.15	0.002	0.398	0.688
TNF-α	Plasma	13	452	367	0.13	−0.31	0.57	0.55	88	103.20	< 0.00001	0.200	0.516
TNF-α	Serum	**9**	**371**	**220**	**0.31**	**−0.00**	**0.61**	**0.05**	**67**	**24.15**	**0.002**	**0.602**	**0.463**
TNF-β	All	7	317	179	−0.12	−0.31	0.07	0.21	0	3.81	0.70	0.764	0.591
TNF-β	Plasma	4	152	93	−0.16	−0.43	0.10	0.23	0	2.05	0.56	1.000	0.519
TNF-β	Serum	3	165	86	−0.08	−0.34	0.19	0.57	0	1.56	0.46	1.000	0.730

et al., 2015; Tostes et al., 2012; Xie et al., 2017; Yang et al., 2015a; Yang et al., 2015b; Zimmerman et al., 2005). There were 1582 male (78%) and 446 (22%) female participants. When authors provided information, the mean age of patients ranged from 3.5 to 17.7 years compared to 3.4–15.4 years in control subjects.

3.3. IFN-γ

Concentrations of IFN-γ were determined in 13 studies (Ashwood et al., 2011b; El-Ansary and Al-Ayadhi, 2012; Guloksuz et al., 2017; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Singh, 1996; Singh et al., 1991; Suzuki et al., 2011; Sweeten et al., 2004; Tobiasova et al., 2011; Tostes et al., 2012; Xie et al., 2017). Overall, blood IFN-γ levels did not differ between patients with ASD (n = 668) and controls without ASD (n = 446) (SMD, 0.26; 95% CI, −0.05 to 0.57; P = 0.10). An I2 = 82% indicated large heterogeneity. Additionally, there was evidence of publication bias (Begg's P = 0.049; Egger's P = 0.016). Subgroup meta-analyses (Table 1) and meta-regression analyses (Table 2) were performed to explore potential sources of heterogeneity and publication bias. The difference in serum IFN-γ levels between patients and controls remained nonsignificant ($P = 0.28$). On the contrary, a SMD of 0.53 revealed that patients with ASD had significantly higher plasma levels of IFN-γ compared to controls ($P = 0.03$) (Fig. 2). Subgroup analysis suggested that the overall heterogeneity and publication bias was largely related to data on plasma measurements of IFN-γ (I2 = 88%; Begg's P = 0.049; Egger's P = 0.020). Supporting this, heterogeneity was reduced to zero and publication bias was not evident on meta-analysis of data on serum measurements of IFN-γ (I2 = 0%; Begg's $P = 0.707$; Egger's $P = 0.815$). Meta-regression analyses showed no effect of difference in the mean age between patients and controls ($P = 0.85$), difference in the percentage of males between patients and

Table 2
Statistics on meta-regression analyses regarding blood concentrations of pro-inflammatory cytokines in ASD.

Meta-regression analyses of blood pro-inflammatory cytokines in ASD										
Moderator	Cytokine	Between-group ASD vs HC	No. of Pairwise	Meta-regression						Proportion of total between-study variance explained
				Coefficient	SE	95% CI		z	*P* value	R^2 analog
Difference in the mean age	IFN-γ	All	12	0.02	0.08	−0.14	0.17	0.18	0.85	−0.15
Difference in the proportion of male subjects	IFN-γ	All	12	−0.04	0.03	−0.10	0.03	−1.11	0.27	−0.07
Publication year	IFN-γ	All	16	−0.03	0.03	−0.09	0.03	−1.10	0.27	0.00
Sample size	IFN-γ	All	16	−0.01	0.00	−0.02	0.00	−1.57	0.12	−0.06
Latitude	IFN-γ	All	**16**	**−0.03**	**0.01**	**−0.05**	**−0.01**	**−2.79**	**0.005**	**0.26**
Difference in the mean age	IL-1β	All	**17**	**−0.52**	**0.19**	**−0.89**	**−0.14**	**−2.72**	**0.007**	**0.26**
Difference in the proportion of male subjects	IL-1β	All	**15**	**0.06**	**0.02**	**0.01**	**0.11**	**2.56**	**0.011**	**0.22**
Publication year	IL-1β	All	20	−0.06	0.04	−0.13	0.02	−1.51	0.130	0.06
Sample size	IL-1β	All	20	−0.00	0.00	−0.01	0.01	−0.53	0.594	−0.14
Latitude	IL-1β	All	20	−0.00	0.01	−0.02	0.01	−0.45	0.651	−0.05
Difference in the mean age	TNF-α	Serum	5	−0.41	0.23	−0.87	0.04	−1.77	0.076	0.42
Difference in the proportion of male subjects	TNF-α	Serum	**4**	**−0.03**	**0.01**	**−0.05**	**−0.01**	**−3.07**	**0.002**	**0.92**
Publication year	TNF-α	Serum	9	−0.09	0.13	−0.34	0.16	−0.69	0.490	−0.09
Sample size	TNF-α	Serum	9	−0.01	0.01	−0.02	0.01	−1.11	0.269	0.22
Latitude	TNF-α	Serum	**9**	**−0.04**	**0.01**	**−0.06**	**−0.01**	**−3.00**	**0.003**	**0.74**

controls ($P = 0.27$), publication year ($P = 0.27$), and sample size ($P = 0.12$). However, there was a significant interaction between the ES and latitude (16 between group comparisons, β = −0.03; 95% CI, −0.05 to −0.01; $P = 0.005$; R^2 analog = 0.26). This indicated that the lower the latitude the more the increase in IFN-γ levels in patients with ASD (Supplementary Fig. 1). Filled meta-analyses were performed to adjust the ES accounting for the small-study effects. In the filled meta-analyses, the difference in blood IFN-γ levels between patients and controls remained nonsignificant (P = 0.124). Moreover, the difference in plasma IFN-γ levels turned nonsignificant (P = 0.894) after "trim and fill" correction (Table 3).

3.4. IL-1β

Pooling data from 17 studies (Ashwood et al., 2011b; Barbosa et al., 2015; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Guloksuz et al., 2017; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Saresella et al., 2016; Suzuki et al., 2011; Sweeten et al., 2004; Tonhajzerova et al., 2015; Tostes et al., 2012; Xie et al., 2017), patients with ASD (n = 704) had significantly higher blood levels of IL-1β than controls without ASD (n = 548) with an ES of 0.35 (95% CI, 0.08 to 0.61; $P = 0.010$). No evidence of publication bias was observed (Begg's $P = 0.183$; Egger's $P = 0.374$) (Fig. 3). However, heterogeneity was high with Q = 92.49 and I2 = 79%. When subgroup analyses were performed, the ES remained significant ($P = 0.05$) and appeared larger (SMD, 0.56; 95% CI, −0.00 to 1.13) for serum IL-1β levels. While it turned nonsignificant for plasma IL-1β levels ($P = 0.06$) associated with a small effect size of 0.2. Heterogeneity was noticeably lower in studies of the plasma IL-1β than in studies of the serum IL-1β (45% vs. 89%).

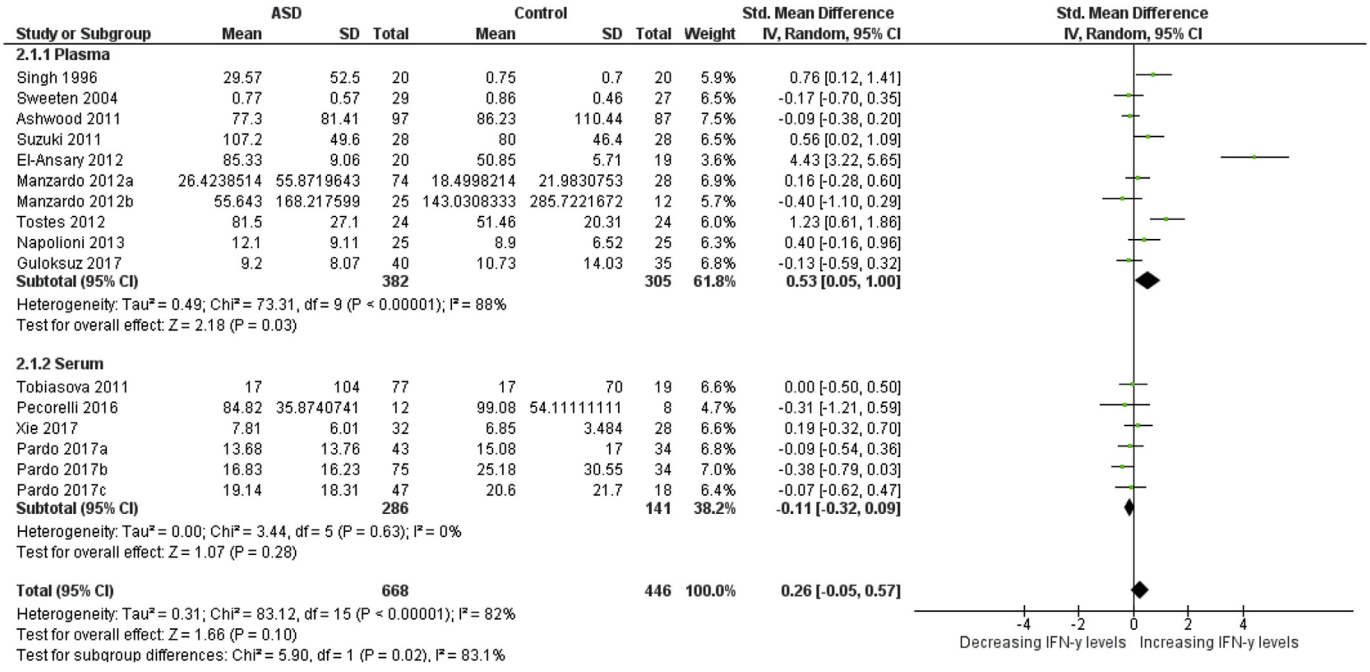

Fig. 2. Meta-analysis of blood IFN-γ levels based on the biomaterial: patients with ASD vs. controls without ASD.

Table 3
Statistics on filled meta-analyses regarding blood concentrations of pro-inflammatory cytokines in ASD.

Filled meta-analysis of blood pro-inflammatory cytokines in ASD						
Cytokine	Between-group ASD vs HC	Pooled Est	95% CI	Z value	*P* value	No. of studies
IFN-γ	All	0.260	−0.05 to 0.57	1.658	0.124	16
IFN-γ	Plasma	0.037	−0.51 to 0.58	0.133	0.894	13
IL-12p40	Serum	0.413	−0.34 to 1.16	1.083	0.279	4

Meta-regression analyses identified a significant negative effect of difference in the mean age between patients and controls (17 between-group comparisons; β = −0.52; 95% CI, −0.89 to −0.14; *P* = 0.007) on the ES of difference in mean levels of blood IL-1β (Supplementary Fig. 2). This represented that the increase of IL-1β levels in patients with ASD was decreased with increasing difference in the mean age between patients and controls. On the contrary, there was a significant positive interaction between the ES and the moderator of difference in the percentage of males between patients and controls (15 between-group comparisons; β = 0.06; 95% CI, 0.01 to 0.11; P = 0.011) (Supplementary Fig. 3). This indicated that the more the percentage of males in patients than that in controls, the more the increase in blood IL-1β levels in patients with ASD compared to controls. Meta-regression results were not significant for other moderators (Table 2).

3.5. IL-6

22 studies provided information on the blood measurements of IL-6 (Ashwood et al., 2011b; Croonenberghs et al., 2002; El-Ansary et al., 2011; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Guloksuz et al., 2017; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Shaker et al., 2016; Singh, 1996; Suzuki et al., 2011; Tobiasova et al., 2011; Tostes et al., 2012; Tsilioni et al., 2015; Xie et al., 2017; Yang et al., 2015a; Yang et al., 2015b; Zimmerman et al., 2005). Random-effects meta-analysis revealed that levels of IL-6 were significantly increased in patients with ASD (n = 857) compared to controls (n = 622) with an overall ES of 0.33 (95% CI, 0.01 to 0.66; *P* = 0.04) (Fig. 4). No publication bias was present (Begg's P = 0.528; Egger's P = 0.602). However, an I2 of 88% reflected the high degree of heterogeneity. In subgroup analyses, I2 dropped to 48% in meta-analysis of serum levels of IL-1β; where the ES remained significant (13 between-group comparisons; SMD, 0.30; 95% CI, 0.07 to 0.52; P = 0.01). On the contrary, I2 was greater (94%) in the subgroup analysis of plasma levels of IL-1β; where the ES turned nonsignificant (P = 0.25). Meta-regression analyses showed no significant effect for any of the investigated moderators on the ES of blood IL-6 levels (Table 3).

3.6. TNF-α

19 studies were entered into the meta-analysis of blood TNF-α levels (Ashwood et al., 2011b; Bryn et al., 2017; El-Ansary et al., 2011; Ghaffari et al., 2016; Guloksuz et al., 2017; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Singh, 1996; Suzuki et al., 2011; Sweeten et al., 2004; Tonhajzerova et al., 2015; Tostes et al., 2012; Tsilioni et al., 2015; Xie et al., 2017; Yang et al., 2015b). Overall, there was no significant difference (*P* = 0.14) in blood TNF-α levels between patients with ASD (n = 823) and controls (n = 587). Publication bias was not detected by Begg's test (*P* = 0.398) and Egger's test (*P* = 0.688). Due to the high degree of heterogeneity (Q = 127.59 and I^2 = 84%), subgroup meta-analyses were performed. The ES remained nonsignificant (*P* = 0.55) and heterogeneity remained at a high level (I^2 = 88%) in the subgroup analysis of plasma levels of IL-1β. In

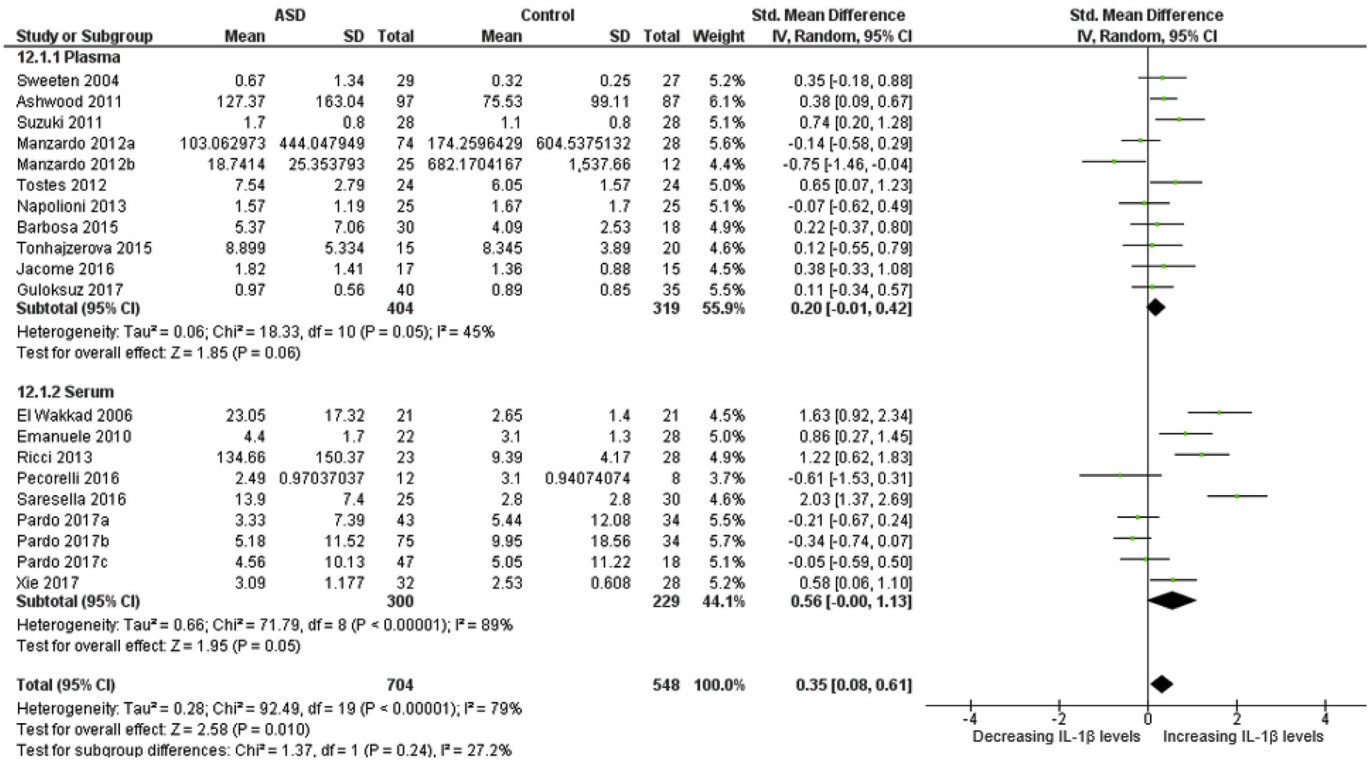

Fig. 3. Meta-analysis of blood IL-1β levels based on the biomaterial: patients with ASD vs. controls without ASD.

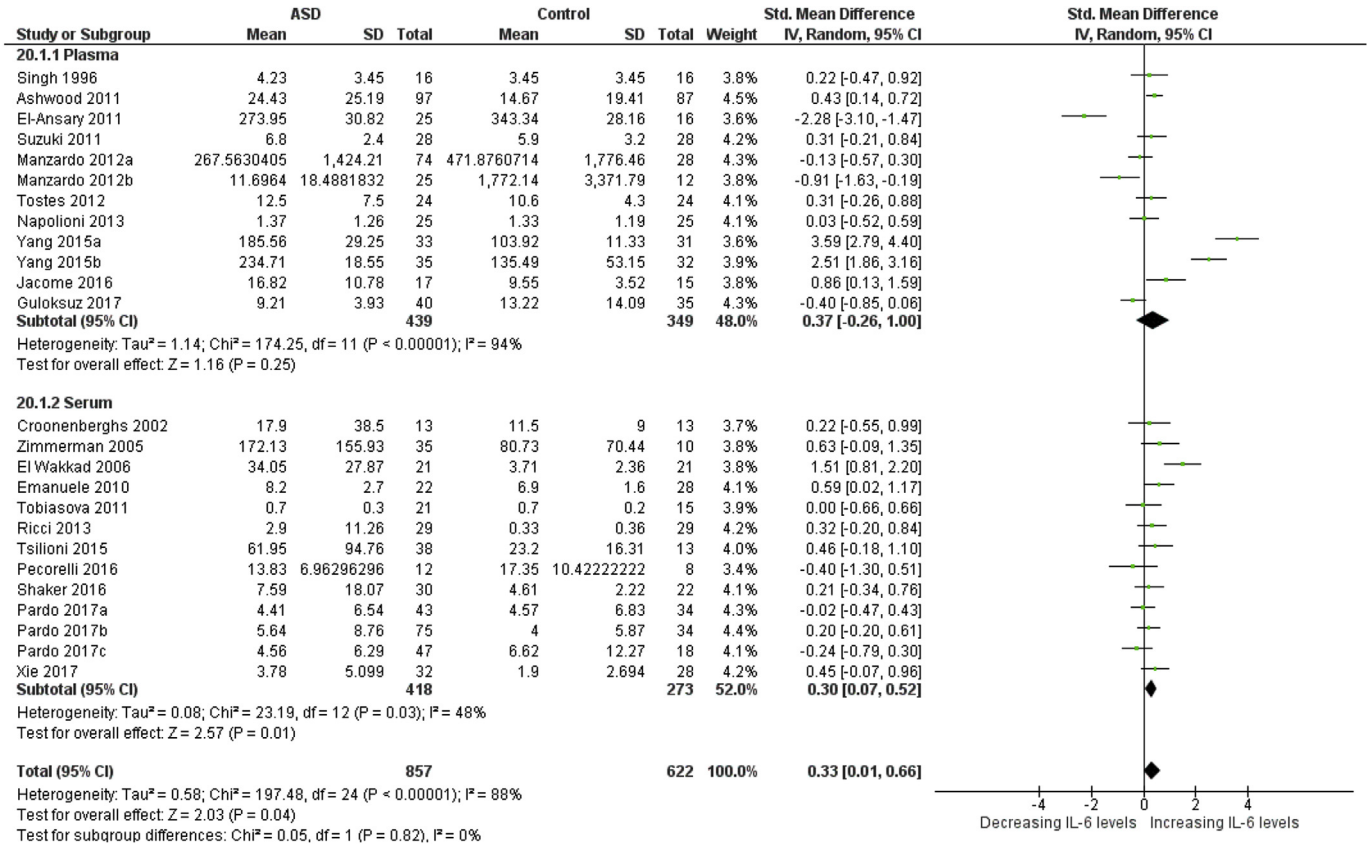

Fig. 4. Meta-analysis of blood IL-6 levels based on the biomaterial: patients with ASD vs. controls without ASD.

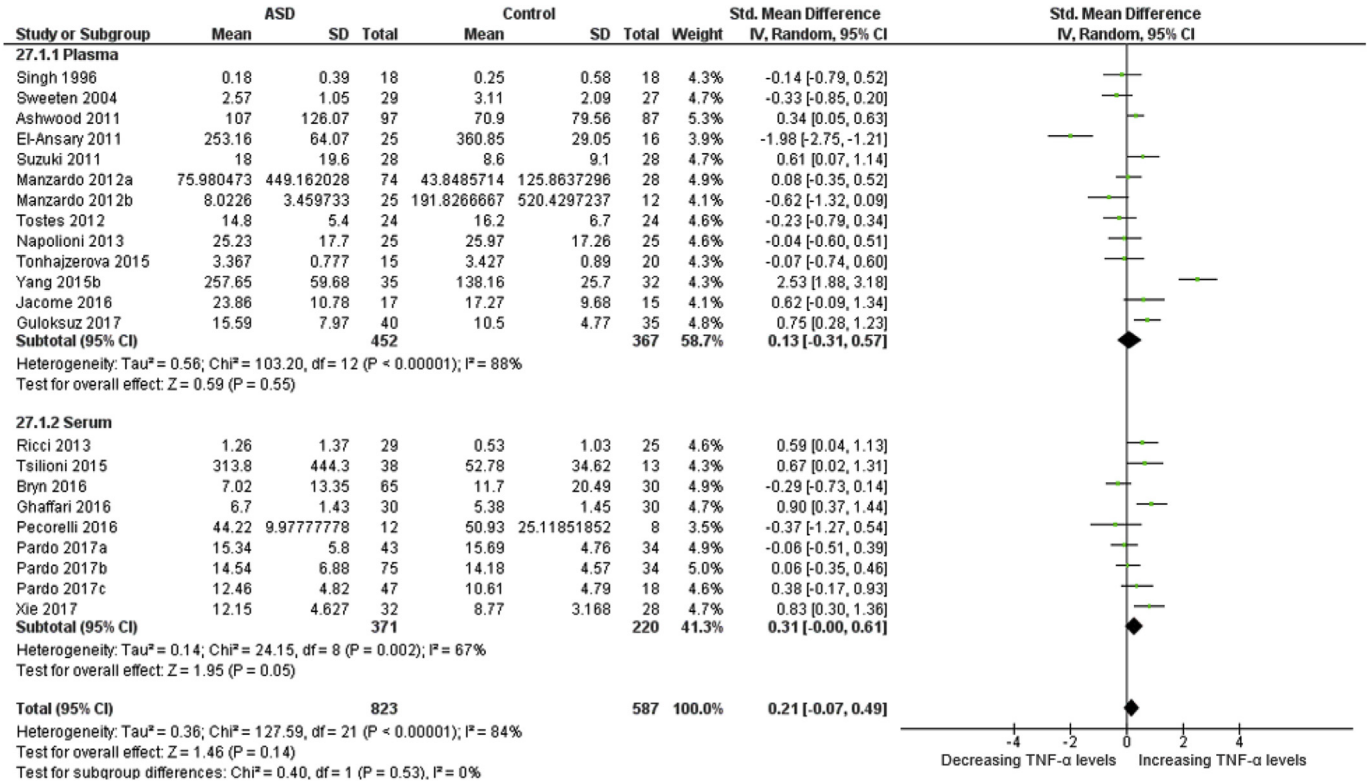

Fig. 5. Meta-analysis of blood TNF-α levels based on the biomaterial: patients with ASD vs. controls without ASD.

contrast, the ES approached significance ($P = 0.05$) in the subgroup analysis of serum levels of IL-1β and the level of heterogeneity was slightly reduced to 67% (nine between-group comparisons; SMD, 0.31; 95% CI, −0.00 to 0.61) (Fig. 5). Therefore, meta-regression analyses were done to further investigate the potential sources of heterogeneity. The moderator latitude was shown to account for about 74% of the between-study variance (nine between-group comparisons; β = −0.04; 95% CI, 0.01 to −0.06; P = 0.003) (Supplementary Fig. 4). This indicates a negative interaction of latitude with the ES of difference in TNF-α level, a result similar to that of IFN-γ levels. Meta-regression analyses also identified a significant effect of difference in percentage of males between patients and controls (β = −0.03; 95% CI, −0.05 to −0.01; P = 0.002) on the ES of difference in serum TNF-α levels (Supplementary Fig. 5). However, due to the low number of studies (n = 4) included in this meta-regression, the result should be interpreted with caution. Other moderators showed no influence.

4. Discussion

The present meta-analysis was conducted to investigate alterations of proinflammatory cytokine levels in patients with ASD compared to controls without ASD. We found medium increases in levels of plasma IFN-γ (SMD = 0.53) and serum IL-1β (SMD = 0.56) and small increases in levels of blood IL-1β (SMD = 0.35), serum IL-6 (SMD = 0.30) and serum TNF-α (SMD = 0.31) for patients with ASD. There were ten between-group comparisons (nine studies) entered into the meta-analysis of plasma IFN-γ. Of these, four (El-Ansary and Al-Ayadhi, 2012; Singh, 1996; Suzuki et al., 2011; Tostes et al., 2012) pointed to higher levels of plasma IFN-γ in patients with ASD and six (Ashwood et al., 2011b; Guloksuz et al., 2017; Manzardo et al., 2012; Napolioni et al., 2013; Sweeten et al., 2004) revealed no association between levels of plasma IFN-γ and ASD. Meta-analysis of IL-1β levels in plasma or serum included 20 between-group comparisons (17 studies); among which eight (Ashwood et al., 2011b; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Ricci et al., 2013; Saresella et al., 2016; Suzuki et al., 2011; Tostes et al., 2012; Xie et al., 2017) linked ASD with an increase in IL-1β levels and 11 (Barbosa et al., 2015; Guloksuz et al., 2017; Jacome et al., 2016; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Sweeten et al., 2004; Tonhajzerova et al., 2015) found no significant difference in IL-1β levels between patients with ASD and controls. Moreover, one between-group comparison (Manzardo et al., 2012) among females showed lower levels of IL-1β in patients with ASD. A total of 25 between-group comparisons (22 studies) including 1479 participants were enrolled into the meta-analysis of blood IL-6 levels. Six (Ashwood et al., 2011b; El Wakkad and Saleh, 2006; Emanuele et al., 2010; Jacome et al., 2016; Yang et al., 2015a; Yang et al., 2015b) of them demonstrated higher levels of IL-6 in patients and 18 (Croonenberghs et al., 2002; Guloksuz et al., 2017; Manzardo et al., 2012; Napolioni et al., 2013; Pardo et al., 2017; Pecorelli et al., 2016; Ricci et al., 2013; Shaker et al., 2016; Singh, 1996; Suzuki et al., 2011; Tobiasova et al., 2011; Tostes et al., 2012; Tsilioni et al., 2015; Xie et al., 2017; Zimmerman et al., 2005) reported no significant alteration in blood IL-6 levels for patients with ASD compared to controls. While, one (El-Ansary et al., 2011) pointed to a decrease of blood IL-6 levels in patients. Meta-analysis of serum TNF-α levels consisted of nine between-group comparisons (seven studies). Of these, four (Ghaffari et al., 2016; Ricci et al., 2013; Tsilioni et al., 2015; Xie et al., 2017) related ASD with increased TNF-α levels and five (Bryn et al., 2017; Pardo et al., 2017; Pecorelli et al., 2016) found no significant association between ASD and serum TNF-α levels. In this manner, our meta-analysis strengthened the clinical evidence provided in individual articles and in the previous meta-analysis reported in June 2014 (Masi et al., 2015).

We found no significant alterations in peripheral levels of other pro-inflammatory cytokines including IL-1α, IL-2, IL-2R, IL-3, IL-7, IL-8, IL-12, IL-12p40, IL-12p70, IL-15, IL-17, IL-18, IL-23, TBF-β, and TNFRI/II in patients with ASD. Masi et al., (2015) reported an increase of IL-8 levels in patients with ASD through a meta-analysis of three studies (290 participants) (Ashwood et al., 2011b; Napolioni et al., 2013; Suzuki et al., 2011). In the present meta-analysis, we pooled data from eight studies (Ashwood et al., 2011b; Bryn et al., 2017; Manzardo et al., 2012; Napolioni et al., 2013; Pecorelli et al., 2016; Suzuki et al., 2011; Tobiasova et al., 2011; Tonhajzerova et al., 2015) (615 participants) and found no significant difference in IL-8 levels between patients with ASD and controls without ASD.

Heterogeneity was high in most meta-analyses. Subgroup meta-analyses based on the sampling source helped lower heterogeneity for cytokines IFN-γ and IL-1β. To further explore potential sources of heterogeneity, we performed meta-regression analyses. Latitude showed a negative moderating effect on the ES of difference in mean levels of IFN-γ and TNF-α. This effect was more pronounced for TNF-α ($R^2 = 0.74$) than for IFN-γ ($R^2 = 0.26$). Moreover, difference in the mean age between patients and controls had a negative interaction with the ES of difference in mean levels of IL-1β. In contrast, meta-regression analyses identified a positive effect for the moderator of difference in the proportion of male subjects between patients and controls on the ES of difference in mean levels of IL-1β. However, this moderator showed a negative influence on the ES of difference in mean levels of TNF-α. This influence should be treated with cation due to small number of observation included in the associated meta-regression.

IFN-γ is a T helper cell 1 (Th1) cytokine with pro-inflammatory effects. Children with autism displayed significant increase in immunostaining for IFN-γ in all cells and in $CD4^+$ T cells as well as in the mRNA expression and protein levels of IFN-γ (Ahmad et al., 2017). PBMCs taken from these patients also revealed an augmented production of IFN-γ against common dietary proteins (Jyonouchi et al., 2002). Of note, there was an enhanced ratio of IFN-γ to IL-10 (Molloy et al., 2006), an indicator of inflammation. Animal studies confirmed upregulation of IFN-γ in animals with autism-like behaviors (Alfawaz et al., 2014; Zhang et al., 2013). More interestingly, women with increased levels of serum IFN-γ at midgestation were significantly more likely to bear a child with developmental delay (Bodnar et al., 2018), especially an ASD (Goines et al., 2011). In contrast, subjects with decreased neonatal levels of IFN-γ were more likely to be diagnosed with ASD, according to study of neonatal dried blood samples (n-DBSS) (Abdallah et al., 2012). While, analysis of brain tissues provided clear evidence of the central increase of IFN-γ in patients with ASD (Li et al., 2009; Patel et al., 2016). Recent research linked high levels of IFN-γ to a reduction in glucocorticoid receptor (GR) levels (Patel et al., 2016). This might result in excessive circulation of glucocorticoids, which are well-known as potent neurotoxins (Croonenberghs et al., 2008).

A prospective cohort of 246 children suggested a significant negative effect for high levels of IL-1β at gestation period on child cognitive abilities and executive functioning (Dozmorov et al., 2018). More precisely, study of NBS revealed that neonatal levels of IL-1β directly increase the risk of development of mild to moderate ASD (Krakowiak et al., 2017). Increased mRNA levels of IL-1β similar to that of IFN-γ showed a negative association with GR levels in the brain tissues derived from autistic subjects (Patel et al., 2016). Prenatal exposure to maternal immune challenge induced by lipopolysaccharide (LPS) and polyinosinic-polycytidylic acid (polyI:C) has been associated with development of autism-like behaviors in rat offspring (Ballendine et al., 2015; Kirsten et al., 2013). Both the mothers and the offspring showed upregulation of IL-1β while the hypothalamus-pituitary-adrenal (HPA) axis apparently remained unchanged. Interestingly, Fingolimod (FTY720) helped improvement of autistic behaviors accompanied a reduction of IL-1β levels in the rat hippocampus (Wu et al., 2017).

TNF-α was among the most common pro-inflammatory cytokines in autistic brains (Li et al., 2009). Along with overexpression of TNF-α, there was hypomethylation of TNF-α (Nardone et al., 2014). Through analysis of amniotic fluid, a Danish birth cohort indicated higher levels of TNF-α and TNF-β in people with ASD (Abdallah et al., 2013). PBMCs

and lymphoblasts from patients with ASD revealed increase in both spontaneous and stimulated (using LPS) production of TNF-α (Jyonouchi et al., 2005a; Jyonouchi et al., 2001; Li et al., 2009). TNF-α production was also increased against common dietary proteins (Jyonouchi et al., 2002). An association between TNF-α production against cow's milk and TNF-α production stimulated by LPS was found in PBMCs from patients with ASD who had gastrointestinal symptoms (Jyonouchi et al., 2005b). This association, however, turned non-significant in people with ASD without gastrointestinal symptoms. Therefore, these lines indicate the importance of TNF-α in pathogenesis of gastrointestinal inflammation in people with ASD. Among 11 cytokines investigated in the study (Xie et al., 2017), TNF-α was the only cytokine associated with severity of ASD symptoms. Linkage study in Italian patients with ASD identified increased transmission of a haplotype comprising the TNF-238(G)-TNF-308(G)-MIB*332-HLA-B*38-HLA-Cw*12 alleles (Guerini et al., 2011). A pilot clinical study by Chez et al., (2012) showed the efficacy and safety of lenalidomide for the treatment of autistic behaviors (Chez et al., 2012). It is interesting that lenalidomide was able to substantially reduce (more than 50%) mean concentrations of TNF-α in CSF and serum. Similarly, improvement in autistic behaviors by treatment with a luteolin-containing dietary formulation accompanied a reduction in TNF-α levels (Tsilioni et al., 2015). Of importance was that the greater the decrease in TNF-α levels the better the improvement (Tsilioni et al., 2015). In a study of the visual system in *Xenopus laevis*, long-term developmental exposure to TNF-α led to the abnormal development of synaptic function (Lee et al., 2010). Synaptic dysfunction is well-documented as an underlying mechanism of neurodevelopmental disorders including ASD (Zoghbi and Bear, 2012). Exposure of mouse brain to TNF-α significantly caused inflammation in the frontotemporal regions responsible for cognitive functions such as learning and memory (Young et al., 2012). Poor memory and learning difficulties commonly occur among people with ASD (Russell et al., 1996; Williams et al., 2006). Moreover, the cytokine TNF-α and its receptor signaling play a crucial role in pathologies related to placental and fetal homeostasis, notably neurogenesis (Carpentier et al., 2011). Administration of curcumin and resveratrol could reduce TNF-α levels while helping to rehabilitate autistic rats (Bhandari and Kuhad, 2015, 2017). Taken together, evidence suggests that the cytokine TNF-α mediates the effects of genetic, epigenetic, and environmental factors in ASD.

PBMCs from patients with ASD with or without stimulation showed an increased production of IL-6 (Enstrom et al., 2010; Jyonouchi et al., 2001; Malik et al., 2011). Also, levels of this cytokine were increased in autistic brains compared to control brains (Li et al., 2009; Wei et al., 2011). More precisely, in vitro analyses of autistic brains revealed that the overexpression of IL-6 may favor the formation of granule cell excitatory synapses (Wei et al., 2011). Supporting this is the fact that ASD is a brain disorder characterized with an enhanced ratio of excitation/inhibition (Rubenstein and Merzenich, 2003). High levels of IL-6 in mouse brain led to the development of behavioral abnormalities resembling ASD in humans (Wei et al., 2012a,b). Maternal immune challenge which is commonly used as a model of ASD will boost levels of IL-6 (Hsiao et al., 2012). Compared to the offspring of wild-type mice, behavioral impairments associated with maternal immune activation were significantly restored in the offspring of IL-6 knock-out mice (Smith et al., 2007). This indicates that the cytokine IL-6 plays a crucial role in mediating the link between maternal immune activation and ASD. Its role seems to be accomplished at least partly by JAK2/STAT3 signaling. Targeting this signaling pathway by treatment with flavonoids such as Luteolin and diosmin significantly ameliorated neurobehavioral problems in the adult offspring exposed to maternal immune activation (Parker-Athill et al., 2009). In addition, increased levels of IL-6 induced by maternal immune challenge would result in aberrations of the growth hormone-insulin-like growth factor (GH-IGF) axis (Hsiao and Patterson, 2011). The overexpression of IL-6 in mouse brain has been associated with enlargement of the brain especially lateral ventricle (Wei et al., 2012a,b). Evidence is conclusive about regional brain enlargement in patients with ASD (Piven et al., 1996) and its association with regressive features as well (Nordahl et al., 2011). Recently, a meta-analysis study pointed to higher erythrocyte concentrations of mercury in people with ASD (Saghazadeh and Rezaei, 2017b). Interestingly, mast cells treated with mercury have been shown to produce higher levels of IL-6 (Kempuraj et al., 2010).

Altogether, the shift to a pro-inflammatory pattern is common among patients with ASD. Recent evidence pointed to the significant impact of such immune profile on the severity of ASD-related symptoms in different domains including learning, social affect, sleep disturbances, and aggression (Careaga et al., 2017). Keeping the above findings in mind, the clinical utility of proinflammatory cytokines e.g. IFNγ, TNFα, IL-1β, and IL-6 for screening and diagnostic purposes seems attractive. In addition, many adjuvant therapies are employed for patients with ASD. Of these, several anti-inflammatory compounds for example Palmitoylethanolamide, celecoxib, flavonoid luteolin, and simvastatin significantly helped to improve behavioral impairments in autistic patients (Asadabadi et al., 2013; Khalaj et al., 2018; Moazen-Zadeh et al., 2018; Taliou et al., 2013). However, there are two few studies that have measured changes in cytokine levels among people with ASD before and after treatment (Chez et al., 2012; Pardo et al., 2013; Tsilioni et al., 2015). Moreover, overall only two studies have assessed cytokine profiles in samples from patients with Asperger syndrome or high functioning autism (Bryn et al., 2016; Suzuki et al., 2011). With such a small number of studies, we were unable to conduct subgroup meta-analysis. Therefore, further research needs to appraise the sensitivity and specificity of ASD-associated pro-inflammatory cytokines for prognostic purposes and for differentiating different types of autism.

5. Concluding remarks

This meta-analysis provided evidence for higher concentration of pro-inflammatory cytokines IFN-γ, IL-1β, IL-6, and TNF-α in autistic patents compared with control subjects. Also, meta-regression analyses pointed to the interaction of latitude, age, and gender with peripheral alterations of associated pro-inflammatory cytokines. Further research needs to appraise the sensitivity and specificity of these cytokines for diagnostic and prognostic purposes.

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Author contributions

Conceptualization: AS, AH, NR.
Data curation: AS, AH, BA, KK, AA.
Formal analysis: AS.
Investigation: AS, AH.
Methodology: AS, AH.
Project administration: AH.
Software: AS.
Supervision: AH, NR.
Validation: AS.
Visualization: AS, AH, BA, KK, AA, NR.
Writing – original draft: AS.
Writing – review & editing: AS, AH, NR.

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Appendix A. Supplementary data

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