Review article

Anti-inflammatory cytokines in autism spectrum disorders: A systematic review and meta-analysis

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ABSTRACT

Background: In the search for the causes of autism spectrum disorders (ASD), inflammatory markers have emerged as potential candidates. The present meta-analysis was performed on studies examining circulating concentrations of anti-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: Twenty-five studies with a total of 1754 participants (1022 patients with ASD and 732 control subjects) were included in the mate-analysis; 4 for interferon (IFN)-α, 9 for interleukin (IL)-1 receptor antagonist (Ra), 9 for IL-4, 6 for IL-5, 3 for IL-9, 14 for IL-10, 7 for IL-13, and 6 for transforming growth factor (TGF)-β. We found a moderate decrease in plasma levels of IL-10 (SMD = −0.59) and a small decrease in serum levels of IL-1Ra (SMD = −0.25) in patients with ASD. On the contrary, serum IL-5 levels were slightly increased (SMD = 0.26) in these patients. We conducted meta-regression analyses to investigate the possible effect of moderatos on the effect size (ES) of difference in mean levels of IL-10. Difference in the mean age between patients and controls showed a negative influence on the ES and was able to explain about 0.4 of total between-study variance. In contrast, latitude exerted a positive effect on the ES and explained a lower proportion (0.1) of total between-study variance.
Conclusions: This meta-analysis provides evidence for the lower concentration of anti-inflammatory cytokines IL-10 and IL-1Ra in autistic patients compared with control subjects. Also, meta-regression analyses point to the interaction of latitude, age, and gender with peripheral alterations of associated anti-inflammatory cytokines.

1. Introduction

Research reveals dysregulation of the cytokine system as a key player of inflammation in neuropsychiatric disorders. This stems from the ability of pro-inflammatory and anti-inflammatory cytokines to go together in a complex interplay with immune cells as well as with messengers of the neuroendocrine system that mainly include neurotransmitters and hormones [1]. More precisely, these cytokines have been shown to interfere with the activity of serotonin transporters [2]

and with diurnal secretion of hormones of the hypothalamic–pituitary–adrenal (HPA) axis [3]. Therefore, it is not surprising that over the last decade, alteration of cytokine levels has been increasingly linked to major neuropsychiatric disorders, particularly depression, schizophrenia, and bipolar disorder [4–6].

Autism spectrum disorders (ASD) are defined as a group of neurodevelopmental disorders characterized by deficits in social communication and social interaction and restricted, repetitive patterns of behavior, interests, or activities [7]. There are many concerns

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surrounding a child with ASD ranging from the high prevalence of comorbidities (learning difficulties [8], sleep disturbances [9], aggressive behaviors [10], and psychiatric disorders [11]) to the lack of definitive diagnosis and treatment. In the search for the causes of ASD, inflammatory markers have emerged as potential candidates. In 2014, Masi et al. [12] published a meta-analysis study and reported the association of some cytokines with ASD. Since this first meta-analysis, numerous studies have determined circulating concentrations of cytokines in people with ASD compared to people without ASD and provided inconsistent results.

Therefore, the update met-analysis was carried out to investigate the difference in blood levels of anti-inflammatory cytokines between patients with ASD and 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 [13–21] and based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [22]. 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 which 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 anti-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 anti-inflammatory cytokines in ASD patients with either (a) healthy control subjects, (b) ASD patients in a different disease stage. Exclusion criteria were: (1) studies that included ASD patients with specific features e.g. chromosomal abnormalities and with other conditions that might affect cytokine measures, (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 [23], the quality of studies included in the between-group meta-analyses was assessed using the Newcastle–Ottawa Scale (NOS) designed for case-control studies [24]. 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 5.7 (SD = 1.2).

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 patients with ASD and HC. As explained in [25], 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
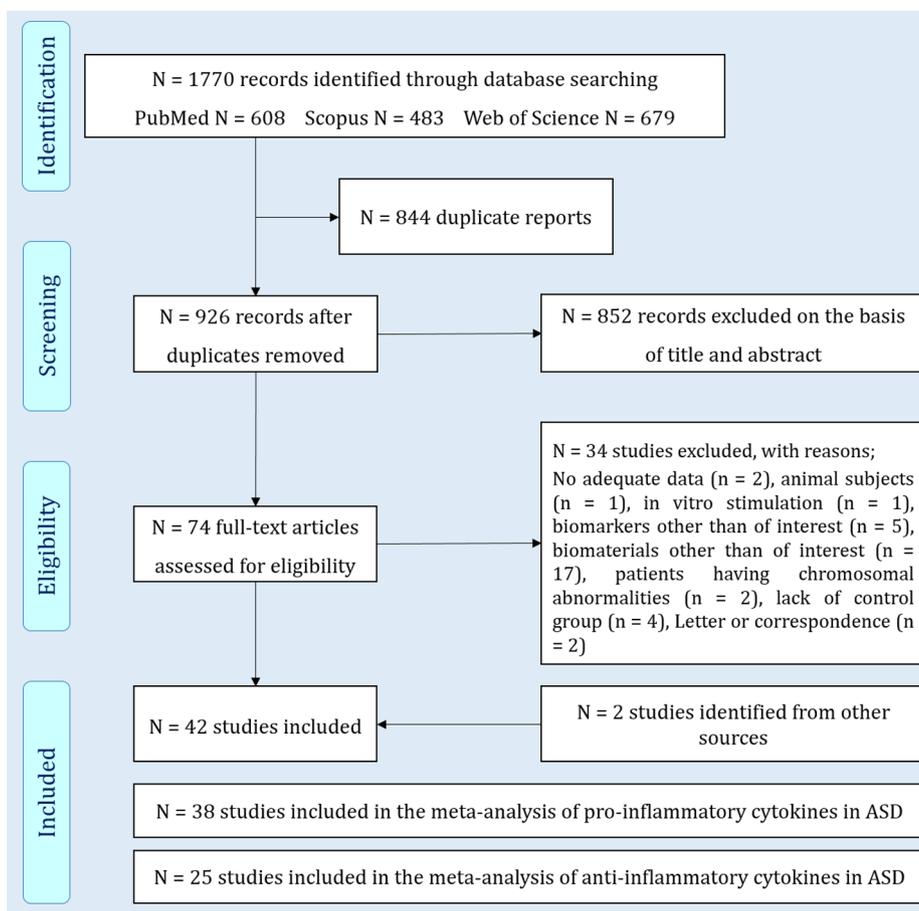
Fig. 1. PRISMA flowchart of study selection for systematic review and meta-analysis of anti-inflammatory cytokines in ASD.

precisely quantitative estimate of heterogeneity. According to the Cochrane guidelines, the less than $40I^2\%$ 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 [26] and Egger bias test [27]. 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 [28].

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, 25 studies with a total of 1754 participants (1022 patients with ASD and 732 controls) were included in the mate-analysis of anti-inflammatory cytokines in ASD [29–53]. With enough data (at least

three between-group comparisons for each cytokine), meta-analysis was conducted for data on nine anti-inflammatory cytokines (IFN-α, IL-1Ra, IL-2Ra, IL-4, IL-5, IL-9, IL-10, IL-13, and TGF-β) 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. Nine studies were conducted in the United States of America [29,32,34,35,38,40,41,52,53], four in Italy [36,37,46,50], two in each of Egypt [43,49], China [30,44], Saudi Arabia [47,48], and Japan [33,39], and one study in each of Cuba [42], Brazil [31], Norway [51], and Turkey [45]. Plasma was used for cytokine measurements in 13 studies [31,33,40–43,45,47–49,52–54], serum in 11 studies [29,30,32,36–39,44,46,50,51], and both plasma and serum in one study [34]. The enzyme-linked immunosorbent assay (ELISA) was used as the analytical procedure for detection of cytokines in most of the included studies (n = 15) [29,34–36,39,40,42–44,46–50,53]. Multiplex techniques were used in seven studies [30,32,33,38,41,51,52], both multiplex techniques and ELISA in two studies [37,45], and both flow cytometry and ELISA in one study [31]. Supplemental information 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 21 studies (84%) [29–31,33-36,38-50,52]. There were studies that included control subjects with similar values for body mass index [45], race [50], ethnicity [33,40], and intellectual functioning [44] as well. There were four studies where patients and controls were matched only for age [37,51,53] or sex [32]. Information regarding the number of male and female subjects was available in most of the included studies [29–36,38–46,48–53]. There were a total of 1315 male (79.6%) and 336 (20.4%) female participants. When information was
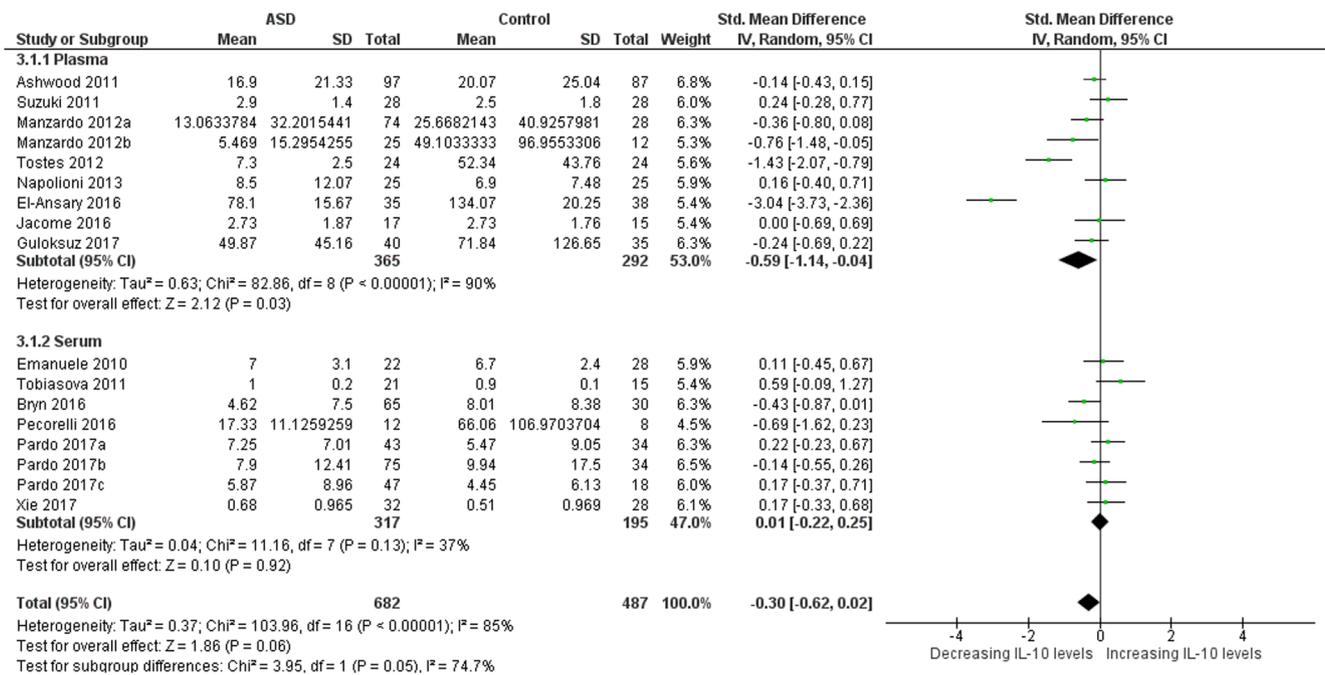
Fig. 2. Meta-analysis of blood IL-10 levels based on the biomaterial: patients with ASD vs. controls without ASD.

provided by authors, the mean age of patients ranged from 3.5 to 28.1 years compared to 3.4 to 28.7 years in control subjects.

3.3. IL-10

Blood levels of IL-10 were extracted from 14 studies [30–33,37,38,40–42,45–47,51,52]. Random-effects meta-analysis demonstrated a trend toward significance for lower blood concentrations of IL-10 in patients with ASD (n = 682) compared to controls without ASD (n = 487) (SMD, −0.30; 95% CI, −0.62 to 0.02; P = 0.06) (Fig. 2). An I^2 of 85% indicated the high degree of heterogeneity. Therefore, subgroup meta-analyses (Table 1) and meta-regression analyses (Table 2) were performed to explore potential sources of heterogeneity. In the subgroup analysis of plasma IL-10 levels, people with ASD were more likely to have significantly lower IL-10 levels than controls without ASD with a medium effect size of −0.59 (nine

Table 1

Statistics on meta-analyses regarding blood concentrations of anti-inflammatory cytokines in ASD.

Group-wise meta-analyses of blood anti-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	I²%	Chi²	P-value	P value of Begg's test	P value of Egger's test
IFN-α	All	7	310	172	0.07	−0.12	0.26	0.45	0	0.7	0.99	0.072	0.158
IFN-α	Plasma	4	145	86	0.10	−0.18	0.37	0.49	0	0.38	0.94	0.308	0.526
IFN-α	Serum	3	165	86	0.05	−0.21	0.32	0.70	0	0.26	0.88	1.000	0.461
IL-10	All	17	682	487	−0.30	−0.62	0.02	0.06	85	103.96	< 0.00001	0.387	0.286
IL-10	**Plasma**	**9**	**365**	**292**	**−0.59**	**−1.14**	**−0.04**	**0.03**	**90**	**82.86**	**< 0.00001**	**0.251**	**0.215**
IL-10	Serum	8	317	195	0.01	−0.22	0.25	0.92	37	11.16	0.13	0.902	0.815
IL-13	All	11	532	321	−0.08	−0.33	0.16	0.50	58	21.51	0.01	0.210	0.286
IL-13	Plasma	5	249	180	0.00	−0.39	0.39	0.99	70	13.19	0.01	0.462	0.944
IL-13	Serum	6	283	141	−0.17	−0.50	0.16	0.30	48	7.63	0.11	0.221	0.074
IL-1Ra	All	12	519	259	−0.07	−0.34	0.20	0.62	66	32.24	0.0007	0.304	0.456
IL-1Ra	Plasma	4	152	93	0.18	−0.46	0.82	0.57	81	15.95	0.001	1.000	0.747
IL-1Ra	**Serum**	**8**	**367**	**166**	**−0.25**	**−0.46**	**−0.05**	**0.01**	**11**	**7.84**	**0.35**	**0.063**	**0.088**
IL-4	All	13	539	376	0.10	−0.07	0.28	0.25	35	18.47	0.25	0.760	0.936
IL-4	Plasma	8	330	254	0.06	−0.18	0.31	0.62	47	13.29	0.07	1.000	0.725
IL-4	Serum	5	209	122	0.18	−0.05	0.42	0.12	4	4.16	0.39	0.806	0.433
IL-5	All	9	426	274	0.18	−0.04	0.40	0.12	45	14.43	0.07	0.754	0.441
IL-5	Plasma	5	249	180	0.12	−0.23	0.46	0.51	61	10.25	0.04	1.000	0.749
IL-5	**Serum**	**4**	**177**	**94**	**0.26**	**0.00**	**0.51**	**0.05**	**1**	**3.02**	**0.39**	**0.734**	**0.646**
IL-9	All	7	304	162	−0.09	−0.29	0.10	0.34	0	5.12	0.53	0.368	0.141
IL-9	Plasma	3	127	68	0.01	−0.29	0.32	0.93	0	0.98	0.61	1.000	0.601
IL-9	Serum	4	177	94	−0.17	−0.43	0.08	0.18	9	3.29	0.35	0.308	0.235
sIL-2Ra	All	5	264	126	0.16	−0.06	0.37	0.15	0	2.52	0.64	0.562	0.015
sIL-2Ra	Serum	3	165	86	0.19	−0.07	0.46	0.16	0	0.53	0.77	1.000	0.422
TGF-β	All	6	216	169	−0.26	−1.28	0.75	0.61	95	92.50	< 0.00001	0.452	0.228
TGF-β	Plasma	4	175	135	0.06	−1.30	1.41	0.94	96	78.43	< 0.00001	0.734	0.138

The row is bold where the related meta-analysis shows statistical significance.

Table 2

Statistics on meta-regression analyses regarding blood concentrations of anti-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	IL-10	All	**13**	**−0.22**	**0.09**	**−0.41**	**−0.04**	**−2.42**	**0.016**	**0.37**	
Difference in the proportion of male subjects	IL-10	All	12	0.01	0.01	−0.02	0.03	0.61	0.541	−0.17	
Publication year	IL-10	All	17	−0.03	0.07	−0.16	0.10	−0.50	0.615	−0.11	
Sample size	IL-10	All	17	0.00	0.01	−0.01	0.01	0.01	0.991	−0.17	
Latitude	IL-10	All	**17**	**0.02**	**0.01**	**0.00**	**0.04**	**2.06**	**0.039**	**0.10**	
Difference in the mean age	IL-10	Plasma	8	−0.30	0.53	−1.34	0.75	−0.56	0.576	−0.18	
Difference in the proportion of male subjects	IL-10	Plasma	8	0.02	0.04	−0.06	0.10	0.46	0.643	−0.24	
Publication year	IL-10	Plasma	9	−0.13	0.13	−0.39	0.13	−0.98	0.326	−0.10	
Sample size	IL-10	Plasma	9	0.00	0.01	−0.01	0.02	0.26	0.798	−0.32	
Latitude	IL-10	Plasma	9	0.02	0.02	−0.01	0.05	1.54	0.124	0.15	

The row is bold where the related meta-analysis shows statistical significance.

between-group comparisons; 95% CI, −1.14 to −0.04; *P* = 0.03). However, the level of heterogeneity still remained high in this subgroup (I^2 = 90%). In contrast, both the impact of ES (*P* = 0.92) and the level of heterogeneity (I^2 = 37%) were reduced to nonsignificant though meta-analysis of serum IL-10 levels. Meta-regression analyses showed the significant negative interaction of moderator difference in the mean age between patients and controls with the ES of difference in blood IL-10 levels (13 between-group comparisons; β = −0.22; 95% CI, −0.41 to −0.04; *P* = 0.016). This represents that the greater the difference in mean age between patients and controls the less the difference in blood IL-10 levels between patients and controls (Supplemental information Fig. S1). In addition, the moderator latitude was found to positively influence the ES of difference in blood IL-10 levels between patients and controls (17 between-group comparisons; β = 0.02; 95% CI, 0.00 to −0.04; *P* = 0.039). This indicates that the greater the latitude the greater the difference in blood IL-10 levels in patients with ASD compared to controls (Fig. S2). As R^2 analog values show, the impact of difference in the mean age was more pronounced than that of latitude (0.37 vs. 0.10). No evidence of small-study effects (publication bias) was present in the overall and subgroup meta-analyses of IL-10 in ASD.

3.4. IL-1Ra

Nine studies provided information on blood IL-1Ra levels [29,32,33,37,38,40,41,50,51]. Overall, people with ASD (n = 519) did not differ (*P* = 0.62) in blood IL-1Ra levels from controls without ASD (n = 259). Heterogeneity was moderate with Q = 32.24 and I^2 = 66%. When subgroup analyses were performed, serum levels of IL-1Ra were significantly reduced in patients with ASD with an ES of −0.25 (eight between-group comparisons; 95% CI, −0.46 to −0.05; *P* = 0.01). Also, heterogeneity turned nonsignificant (I^2 = 11%) in the subgroup of serum IL-1Ra levels (Fig. 3). Whereas, the impact of ES remained nonsignificant (*P* = 0.57) and heterogeneity was higher (I^2 = 81%) in the subgroup analysis of plasma IL-1Ra levels. There was no evidence of publication bias in the overall analysis and subgroup analysis of plasma IL-1Ra levels. Evidence of publication bias, however, appeared in the subgroup analysis of serum IL-1Ra levels (Begg's P = 0.063; Egger's P = 0.088). Therefore, filled meta-analysis was performed to adjust the ES accounting for the small-study effects (Table 3). The ES still remained significant after "trim and fill" correction (*P* = 0.001).

3.5. IL-5

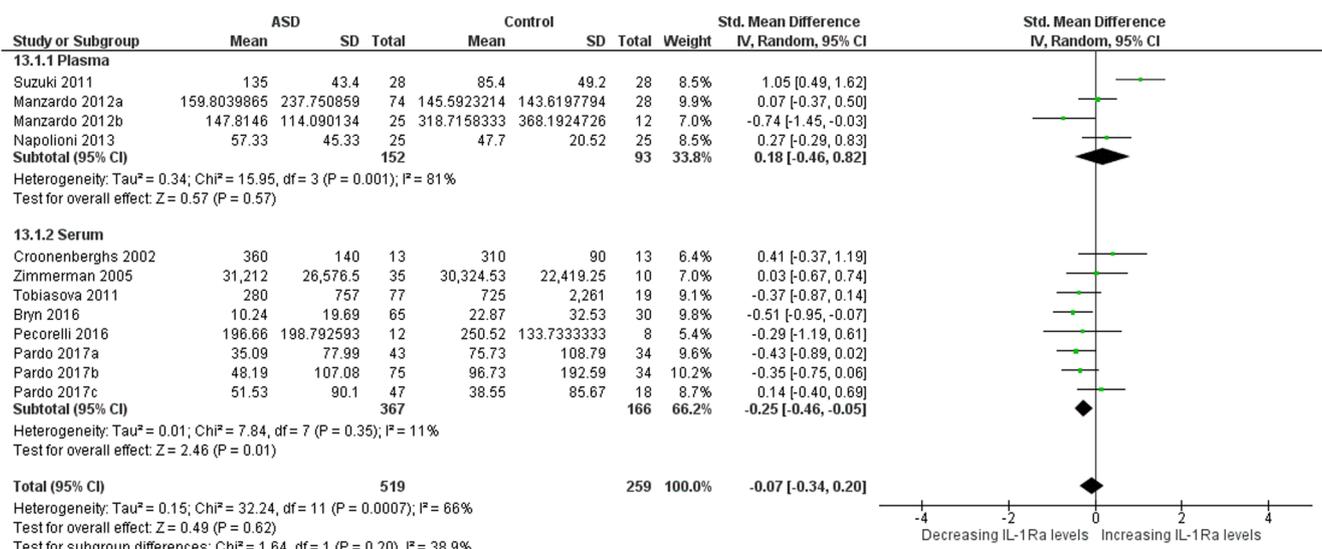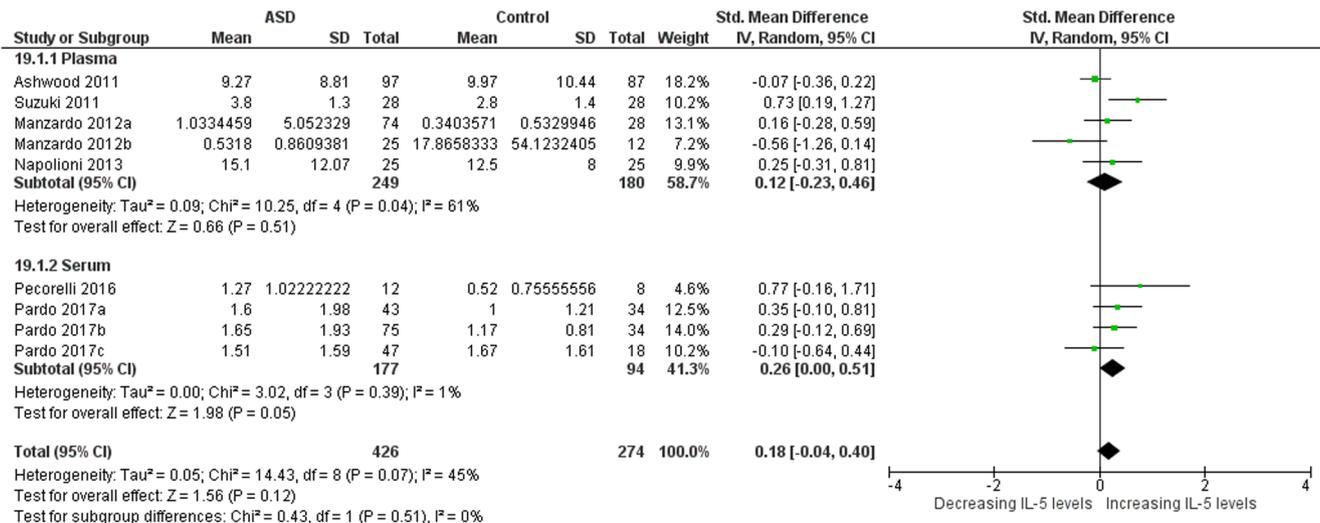Pooling data from six studies [33,37,38,40,41,52], blood IL-5 levels

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

Table 3

Statistics on filled meta-analyses regarding blood concentrations of anti-inflammatory cytokines in ASD.

Filled meta-analysis of blood anti-inflammatory cytokines in ASD						
Cytokine	Between-group ASD vs HC	Pooled Est	95% CI	Z value	P value	No. of studies
IFN-α	All	0.052	−0.13 to 0.23	0.575	0.565	9
IL-13	Serum	−0.171	−0.50 to 0.16	−1.027	0.305	5
IL-1Ra	**Serum**	**−0.304**	**−0.49 to −0.12**	**−3.257**	**0.001**	**9**

The row is bold where the related meta-analysis shows statistical significance.

Fig. 4. Meta-analysis of blood IL-5 levels based on the biomaterial: patients with ASD vs. controls without ASD.

were not different ($P = 0.12$) between patients with ASD (n = 426) and controls without ASD (n = 274) (Fig. 4). An I^2 of 45% suggested the presence of low level of heterogeneity. The level of heterogeneity increased to 61% and the ES remained nonsignificant in the subgroup analysis of plasma IL-5 levels ($P = 0.51$). On the contrary, the heterogeneity was nearly abolished ($I^2 = 1\%$) in the subgroup analysis of serum IL-5 levels, where the ES of 0.26 (95% CI, 0.00 to 0.51) approached significance ($P = 0.05$).

4. Discussion

In this update meta-analysis, we aimed to determine whether blood cytokine profile in people with ASD is different from that in people without ASD. We found a moderate decrease in plasma levels of IL-10 (SMD = −0.59) and a small decrease in serum levels of IL-1Ra (SMD = −0.25) in patients with ASD. On the contrary, serum IL-5 levels were slightly increased (SMD = 0.26) in these patients. Then, we performed subgroup meta-analyses to explore potential sources of heterogeneity. Interestingly, subgroup meta-analyses based on the sampling source (plasma and serum) helped to substantially reduce heterogeneity across studies providing data for cytokines IFN-α, IL-4, IL-5, IL-10, and IL-1Ra. Finally, we conducted meta-regression analyses to investigate the possible effect of moderatos on the ES of difference in mean levels of IL-10. Difference in the mean age between patients and controls showed a negative influence on the ES and was able to explain about 0.4 of total between-study variance. In contrast, latitude exerted a positive effect on the ES and explained a lower proportion (0.1) of total between-study variance.

Of eight studies included in the present meta-analysis of plasma levels of IL-10, two [41,47] found lower levels of IL-10 in people with ASD than controls without ASD and six [31,33,40,42,45,52] found no association between plasma levels of IL-10 and ASD. Studies of peripheral blood and mucosal biopsies showed that the number of CD3$^+$IL-

10$^+$ cells is decreased in patients with ASD not only compared to non-inflamed controls [55] but also compared to controls who had inflammatory bowel disease [56]. Consistently, PBMCs from patients with ASD and gastrointestinal symptoms showed a lower production of IL-10 than patients with ASD and without gastrointestinal symptoms [57]. Lower expression levels of IL-10 correlated with the MET gene C allele as well as with lower levels of MET protein, which both have been implicated in the presence of autism-associated maternal auto-antibodies to fetal brain proteins [58]. The Danish newborn study also revealed that reduced neonatal levels of IL-10 were more common among children who later developed ASD [59]. Surprisingly, a birth cohort though analysis of amniotic fluid indicated that people with ASD were more likely to have higher levels of IL-10 than controls without ASD [60]. However, analysis of brain tissues pointed no significant difference in IL-10 levels between patients with ASD and controls [61]. Of clinical importance is the protective effect of IL-10 on autism-like behaviors associated with maternal immune challenge [62]. It should be noted that this effect depends on the balance between pro-inflammatory and anti-inflammatory cytokines. In fact, excessive amounts of IL-10, which in turn were linked to behavioral abnormalities [63], can be neurotoxic [64].

There were only four between-group comparisons included in the meta-analysis of serum IL-5 levels. None of them showed any association between this cytokine and ASD. PBMCs from patients with ASD produced more IL-5 than PBMCs from controls without ASD [65,66]. In addition, women with higher levels of serum IL-5 at midgestion were significantly more likely to bear a child who later develops ASD [67]. However, analyses of brain tissues and amniotic fluid found no significant difference in brain IL-5 levels between patients with ASD and controls without ASD [60,61]. An interesting finding revealed by an open-label study [68] is that clinical responders had an increase in the mean value of the plasma IL-5 levels following treatment with risperidone. Whereas, clinical nonresponders showed a reduction in the mean

value of the plasma IL-5 levels.

Finally, six [29,32,37,38,50,51] studies were entered into the meta-analysis of serum levels of IL-1Ra. All of them except one [51] failed to find a significant association between serum IL-1Ra levels and ASD. Less in known about the importance of IL-1Ra in immunopathogenesis of ASD.

In the first meta-analysis study reported by Masi and colleagues [12], there were only three studies included in the meta-analysis for each of IL-10 [31,33,40] and IL-1Ra [33,40,50]. Moreover, the authors did not perform a meta-analysis of studies providing data on IL-5 levels. To our knowledge, this is the first meta-analysis study pointing to the peripheral alterations of IL-10 and IL-5 in people with ASD. Pooling data from greater number of studies, we rejected the increase of IL-1Ra levels in people with ASD as revealed by Masi et al. [12]. Instead, we found a significant decrease of serum IL-1Ra levels in patients with ASD. In addition, the previously reported meta-analysis demonstrated a negative association between ASD and TGF-β levels. There were three studies [39,43,53] included in the associated meta-analysis. Here, we performed a meta-analysis of six studies and rejected any association between ASD and TGF-β levels. In this manner, our meta-analysis strengthened the clinical evidence provided in individual articles and in the previously reported meta-analysis.

The role of inflammation has been implicated in a variety of pathologies that occur outside as well as inside the brain. Most often, the link of inflammation with these pathologies is correlative, not causative. In spite of this, interest in establishing such correlations and strengthening the previously reported correlations is exponentially increasing. The main driving force behind this interest is that there are many available inflammatory markers that can easily be measured and be targeted. This force along with the findings of the present meta-analysis proposes IL-10, IL-1Ra, and IL-5 as potential biomarkers of ASD. An idea for future clinical studies is to examine treatment-associated changes in peripheral concentrations of these cytokines among people with ASD. It would be further interesting to investigate the effects of compounds that might help to normalize the balance between pro-inflammatory and anti-inflammatory cytokines, either by increasing the levels of pro-inflammatory cytokines or by decreasing the levels of anti-inflammatory cytokines.

5. Concluding remarks

This meta-analysis provided evidence for lower concentration of anti-inflammatory cytokines IL-10 and IL-1Ra 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 anti-inflammatory cytokines.

CRediT authorship contribution statement

Amene Saghazadeh: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Bahar Ataeinia:** Data curation, Visualization. **Kimia Keynejad:** Data curation, Visualization. **Amirhussein Abdolalizadeh:** Data curation, Visualization. **Armin Hirbod-Mobarakeh:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Visualization, Writing - review & editing. **Nima Rezaei:** Conceptualization, Supervision, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.154740.

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