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Prevalence of Asthma in Children with Autism and Attention Deficit Hyperactivity Disorder

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ABSTRACT

Background: Inflammatory reaction and asthma in certain children with features of autism spectrum disorders (ASD) as well as attention-deficit hyperactivity disorder (ADHD) have been reported previously.

Aim of the Work: Our primary goal in conducting this study was to look into how childhood asthma and attention deficit hyperactive disorder and childhood autism are related.

Patients and Methods: Childhood ASD and ADHD patients' peripheral immune cells were removed, and after ex vivo mitogens were activated, the generation of the cytokines IL-17, IL-13 and IL-4 was evaluated. All ASD and ADHD individuals fulfilled the requirements for identification according to the Diagnostic and Statistical Manual of Mental Disorders, fifth Edition (DSM-V). The level of autism was assessed using the Childhood Autism Rating Scale (CARS). In order to ascertain the correlation between asthma and ADHD symptoms, generalized estimating equations were employed.

Results: Importantly, in contrast to children with ASD as well as ADHD produced considerably more IL-17 after stimulation. Additionally, individuals with ASD, ADHD and co-morbid asthma had higher levels of IL-17.

Conclusion: When T cells were stimulated, children with ASD and ADHD responded differently producing more IL-17.

Keywords: ADHD; Asthma; Autism; Food allergies; IL-17.

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INTRODUCTION

The term "autism spectrum disorder" (ASD) relates to a variety of socially characterized diseases that are varied in nature [1]. Affected people with ASD frequently exhibit stereotyped behaviours and have narrow interests. ASD currently affects 1 in 68 children, with boys being affected at a rate of 1 in 42 [2]. Presently, there are no genetic polymorphisms that all people with ASD share. Although the exact reasons of ASD are unknown, it has been proposed that a variety of aetiologies, especially gene X environment interaction, may contribute to the disorder [3]. The immune reaction can contribute to the aetiology and/or ontogeny of ASD, according to an increasing body of researches. According to earlier research, many children with ASD exhibit dysregulated immunological responses, including elevated activities of natural killer cells, the existence of autoantibodies against brain proteins, and changed cytokine patterns [4]. Researchers have also discovered that a number of immune-mediated illnesses like asthma, allergies, and skin conditions like eczema and atopic dermatitis, may be connected to autism [5-7].

Family relatives of ASD children also have a higher incidence of immune-mediated diseases. Concerning the occurrence of clinically significant immune-mediated diseases and whether they accurately represent immunological dysregulation in ASD or not, there are contradictory findings among study results [8].

Children frequently experience asthma and attention-deficit/hyperactivity disorder (ADHD) both are chronic conditions. Asthma, which is characterized by wheezing, dyspnea, and a chest tightness that affects about 6-8% of children and teenagers [9]. A meta-analysis and other earlier studies have found a connection between asthma and ADHD symptoms [10], even though there are times when a correlation was not found [11]. In one study of 102.253 children from the National Survey of Children's Safety, patients with asthma used to have a two times increased prevalence of ADHD, while children who suffered severe asthma exhibited an even greater rate [2]. Evaluations of general behavioral issues have also revealed comparable dose-response correlations [12].

It's unclear exactly how asthma and ADHD are related to each other. Since hyperactivity-impulsivity (HI) and inattention (IN) have partially different etiologies and developmental consequences [13], no earlier investigations have made a distinction between the two Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V) ADHD diagnostic categories [14]. All cross sectional previous research indicating a correlation between ADHD and asthma has limited the ability to examine the degree of influence and evolutionary connections between the two disorders [15]. Additionally, further research must be done on potential mechanisms that underlie this connection.

First, there may be a connection underlying asthma and ADHD due to common factors associated [16]. Asthma and ADHD are established potential risks for lower birth weight, poor fetal growth, and lower socio-economic status [17]. The majority of prior research on the link between ADHD and asthma has corrected for gender and several socio-economic position variables, with findings remaining constant. However, neither of the investigations have accounted for low birth weight [4].

Furthermore, asthma treatments including inhaled corticosteroids could be blame for the connection between asthma and ADHD (ICS). In young children receiving ICS, there have been published studies of changed behavior and psychotic [18], but broader research shows no proof of significantly negative neuropsycho-logical consequences [19]. One research was capable of demonstrating elevated incidence of hyperactivity in asthmatic children when contrasted to unaffected controls, but the levels were equivalent to other children receiving specialized care for no respiratory problems, showing that ICS has no behavioral effects on children with asthma [20].

Thirdly, the apparent link between asthma and ADHD may be explained by genetic characteristics that run in families. Though studies have repeatedly demonstrated that both asthma and ADHD are hereditary illnesses [21], research on the genetic and cultural factors that may contribute to the two diseases' overlapping is still missing. Two earlier family-based researches looked at how hereditary influences might affect the connection but were unable to establish such a finding [22]. Obviously, more investigation is required to shed light on this problem.

It is thought that IL-17 is generated by Th17 CD4+ cells, which have been shown to produce IL-22, IL-21, IL-17F and IL-17. It has been shown that people with asthma have greater plasma levels of IL-17 [23]. Additionally, there is conflicting evidence concerning IL-17 levels in patients with ASD, which is probably a result of the use of various methodologies, varying data analysis sample sizes, or various research approaches, such as population-based studies as contrasted to researches done out in specialised clinics [24-26]. Several research found no significant disparities in IL-17 concentrations in plasma or serum or publications found elevated serum concentrations of IL-17 in people with ASD [27]. According to one research, IL-17 generation was similar in children with ASD and control children with the same age, but there was a tendency for more frequent IL-17 that created particular Th17 cells in ASD patients [28].

The main objective of this study was to recognize whether there was a connection between the co-morbid characteristics of asthma, allergies, and eczema and the cellular generation of the cytokines IL-4, IL-13, and IL-17 in ASD and ADHD subjects. In order to achieve this, we looked at cellular IL-17 generation after stimulating, allergy/asthma related to Th2 cytokines, IL-4 and IL-13, in children with ASD and ADHD.

PATIENTS AND METHODS

Study design and duration

This was a population-based retrospective observational survey performed at outpatient clinic, Al-Azhar University Hospitals, Egypt from June 2021 to August 2022.

Study participants

A. Inclusion criteria

Children with ASD or ADHD were both participated in this research. Children had to live with at minimum one birth parent and be aged between 5 and 12 years to be qualified to participate in the research.

B. Exclusion criteria

Children with background of developmental milestone delays, lacking parental atopy, or any psychological or neurological illnesses.

Data collection

The survey included 150 children with ASD and ADHD. The developmental milestones, the beginning of complaints, the background of vaccinations, the diet, the experience of sleep disturbances, the indications of the gastro-intestinal tract, and the history of recurrent infections have all been meticulously documented. Pedigree development up to three generations was carried out, with a focus on relatives who were similarly affected as well as other related diseases. In line with the DSM-V, all ASD and ADHD participants satisfied the clinical definition for ASDs and ADHDs. The Childhood Autism Rating Scale (CARS) was used to rate the degree of autism. Every child had electroencephalography been done. Patients were defined as asthma when the airways narrow and swell and may produce extra mucus. This can make breathing difficult and trigger coughing, a whistling sound (wheezing) when patient breathe out and shortness of breath.

CARS and DSM-V

There are 15 elements in the CARS, and each one counts similarly towards the final score. Half of each element is scored, with scores ranging from 1 to 4 [29]. Psychiatry physicians grade the scale based on data gleaned through examinations of children and family interactions. interpersonal interactions, imitations, emotional reactions, body use, item use, change adaptation, visual responses, auditory responses, taste and smell responses, touch use, fear/nervousness, verbal and nonverbal communication, levels of activities, levels of intellectual responses, and overall impressions are the scale's elements. A grade of 15 to 29 indicates that the youngster does not suffer from autism, 30 to 36, mild to moderate autism, and 37 to 60, severe autism [30]. Children with ASD were divided into two sections based on their CARS overall scoring: those with mild-to-moderate ASD and those with severe ASD.

Using a questionnaire of 14 DSM-V-based elements, parents rated the severity of their kid's ADHD symptoms and indicated whether they had persisted within at least six months (0/1). Eight DSM-V indications of hyperactivity-impulsivity were added up to generate a hyperactivity-impulsivity scaling score, and six items linked to attention deficit were added to produce an attention problems measure. Cut-offs were prescribed to allow different

compartmentalised factors that recognize children with (a) one or much more HI/IN signs (HI-1 and IN-1) [31], (b) two or maybe more HI/IN symptomatology (HI-2 and IN-2), and so on until (f) six or more HI/IN diagnoses (HI-6 and IN-6) because there are compelling arguments for contemplating ADHD as an extraordinary of a geometrical characteristic [32, 33].

Pulmonary Function Data

Each child underwent PF testing in the morning or early afternoon by the paediatric physician. Using a rolling-seal spirometer (Spiroflow; P.K. Morgan Ltd., Gillingham, USA), each kid was instructed to execute up to seven maximum forced expiratory flow-volume procedures [34]. FVC, FEV1, maximum midexpiratory flow (MMEF), forced expiratory flow rate at 75% of expired FVC (FEF75), and peak expiratory flow rate were then derived from the data (PEFR). Using flow-volume syringes, the six spirometers used in the study had their calibrations checked before, during, and after each day's testing (Jones Medical Instrument Co., Oak Brook, IL). Information about the data management and quality assurance practises was previously provided [34].

Immunology Challenges and PBMC (Peripheral Blood Mononuclear Cells) Extraction

Blood samples were gathered in acid-citrates-dextrose sterile tube (BD Biosciences, San Jose, USA). Plasma was extracted from samples after 2,300 rpm of centrifugation. For the purpose of isolating PBMC, the buffy coating layer was increased to 20 ml using Hank's Balancing Salt Solution (HBSS), Pennsylvania, deposited over 15 ml of Histopaque-1077 (Hb-1077), and centrifuged at 1,700 rpm for thirty minutes. Trypan Blue was used to quantify the viable PBMC after two HBSS washes of the separated PBMC.

With a mixture of serum-free X-Vivo medium mixed with 0.2% T-stim, PBMC had increased to a dose of 3×10^6 viable cells ml -1. In 96-well tissue culturing plates, 100 microliters of PBMC suspensions were seeded into 100 µlitres of 10 l/ml phytohemagglutinin (PHA) or 100 litres of medium. Following a 48-hour incubation period at 37 °C for the cells, the supernatant was collected and kept at 80 °C for cytokines testing.

Cytokine Measurements

IL-17, IL-13, and IL-4 were among the cytokines/chemokines that were examined via Luminex-based multiplexing platforms employing the Multi-Plex beads. Subsequently, overnight at 4 °C on a rotary shaker with in darkness, analyte-specific antibodies attached particles were treated with 25 µl of assays buffers, 25 µl of X-vivo, and 25 µl of the cultured cells effluent. The liquid was softly sucked by vacuum manifolded aspirations the next day once the plates had warmed to room temp, and the beading had been cleaned. Then, 25 µl of detecting antibodies were applied to each well, and they were shaken for an hour at room temperatures. Each well was then filled with 25 µl of Streptavidins-Phycoerythrins, which was then agitated for thirty minutes at room temperature on plates shaking. Following three rounds of washing, 150 µl of 1 × Sheath liquid, was injected to each well, and the fluorescence was then evaluated using a Bio-Plex 200 Structure.

Impulse oscillometry (IOS)

The IOS system, manufactured by Care Fusion in Yorba Linda, California, was validated using a number of full stroking of a single air volume (3 L) at various flows, which were confirmed using a standard resistance instrument that the manufacturer provided (2.0 cmH2O/L/sec). Beginning the night before assessment, respondents stopped using both short-acting and long-acting bronchodilators. Testing was carried out and its results were evaluated in line with ERS/ATS recommendations [35, 36].

The youngster was seated in order to conduct IOS, and was told to breathe normally and put their lips around the IOS pneumotachometer's mouthpiece. The cheeks were clipped with a nasal clipping and the kid's or parent's hands were positioned there to prevent them from swelling further. Resistance (R), the energies needed to move the pressures waves throughout the airways, and reactance (X), which represents the viscoelastic characteristics of the respiratory system, were used to determine and quantify lung resistance. The average scores of reactance and resistance for rates from 5 to 20 Hz, namely R5, R10, R20, and X5, were computed from a 30-sec (minimal) interval of measurement. Also examined was the region of reactance (AX), which shows the accumulation of reactance

levels beneath resonant frequencies. Providing the coherence, a test reliability indicator, was satisfactory at (>0.80 at 10 Hz) [37, 38] the study was successful due to the absence of indication of coughing, swallowing, vocalisation, or breath holding. Three suitable observations on mean were assessed and visually represented. When determining airways reversibility, a broncho-dilator was given (two inhalations of albuterol, 90 mcg) while utilising spacers. IOS was performed again after 15 minutes. The CV, a measure of trial-to-trial variation and a measure of testing repeatability, is calculated by the IOS equipment. The accuracy of the reaction was then assessed by comparing the bronchodilator reaction to the pre- and post-test responses CV. The manufacturer recommendations baseline standard levels for the instrument based on current reference standards were used to estimate values for R and X in relation to gender and size [39, 40].

Ethical considerations: A written consent was taken from each child parent or legal guardian. Approval was taken to conduct this research from Al-Azhar University Hospitals, Egypt. When performing this human study, the World Medical Association's code of ethics known as the Declaration of Helsinki was fulfilled.

Statistical Analysis: The incidence of immune-mediated conditions in the ASD, ADHD was measured using Fisher's exacting test to assess whether a significant statistical distinction existed. Owing to the skewing nature of the dataset, the medians and range intervals of cytokines levels (pg/ml) were shown on a $\log_{10}$ Y-axis. A two-tailed Mann-Whitney U testing was used to compare the cytokine levels between the ASD and ADHD. The value of LOD/2 was assigned for cytokine levels that were lower than the limit of detection [41]. For IL-17, IL-13, and IL-4, contrasts between diagnostic groups, ASD, and ADHD were first made using graphing and statistics. After that, the concentrations of IL-17, IL-13, and IL-4 secretion were analysed within and between diagnostic cohorts with and without immune-mediated disease. For an association among IL-17, IL-13, and IL-4 cytokine concentrations triggered by PHA and autism intensity ratings, the spearman correlation coefficient (r) was determined. P-values were calculated using a Mann-Whitney U t-test with two tails (p significant if <0.05).

RESULTS

Patient's general characteristics

According to the current findings, males to females with ASD and ADHD were preserved at a ratio of 4:1 with a mean age of 6.98 years old (Table 1).

Impulse oscillometry

Participants having numerous appointments were randomised to choose one appointment for the primary analysis.

Pre-bronchodilator measurements have been performed on all participants using standard baseline data for IOS did not reveal any signs of airflow limitation, and those with and without asthma did not vary significantly from one another given that those with asthma were receiving treatment. Furthermore, the results showed a statistically significant disparity in the percentage differences of R5, R10, X5, and AX, with R10 being the most notable, P = 0.0008 (Table 2) among individuals with asthmatic contrasted to those without respiratory problems.

R10, which displayed the greatest AUC (0.7147) and was preceded by AX (AUC = 0.6639), had the optimal profiles for diagnosis of clinical asthma applying ROC amongst the IOS variables statistically meaningful for bronchial responses (Fig. 1A). The optimal cut-offs value for R10 was discovered to be -8.6%, which had the highest value of sensitivity and specificity (Table 2).

At this stage, IOS had a 77% sensitivity and 76% specificity for accurately diagnosing children with asthma against others without asthma. The cut-offs values for AX was -29.1%, which corresponds to a sensitivity and specificity of 67% and 69%, respectively.

Abnormalities with immune mediation in survey respondents

ADHD patients represented (7.4%), while cases with ASD (27.3%) had a substantially higher reported prevalence of asthma (p=0.025). In this report's subjects, the incidence of the other immune-mediated disorders examined did not differ significantly from that of asthma (Table 3).

Elevated amount of stimulated IL-17 in ASD patients

Once stimulated with the wide T cell activating PHA, T cells separated from peripheral circulation may develop into Th2 and Th17 cells, generating IL-4 and IL-13 (Th2) or IL-17 (Th17) (Th17) [42]. So, applying Luminex methodology to ascertain whether there were any variations in the generation of IL-17, IL-13, or IL-4 from stimulated PBMC comparing ASD and ADHD participants. There were no discernible variations in the IL-17, IL-13, and IL-4 basal concentrations (media only) between the ASD and ADHD populations. Supernatants from PBMC culturing treated with PHA were discovered to significantly enhance the generation of IL-17 in ASD children (87.24 pg/ml, 8.67 pg/ml to 1,689 pg/ml, p = 0.054) when contrasted to ADHD patients (median (55.88 pg/ml, 0.199 pg/ml to 4,751 pg/ml; p=0.036) (Figure 2-A). There were no significant differences in IL-13 and IL-4 release between patients with ASD and ADHD (Figure 2-B and 2-C).

Cytokine concentration in asthmatic patients

After that, we looked to see whether there was any connection between the release of IL-17, IL-13, and IL-4 in Individuals with ASD and ADHD counterparts and immuno-mediated diseases such eczema, allergies and asthma. Environmental (seasonal fevers and other hazards like mould), food, pharmaceutical, skin (except eczema), and skin (including eczema) allergies were further broken down. Additionally, diagnostic categories were assessed for any immune-mediated disease or allergies. The choice of these specific immune-mediated illnesses was primarily based on their accessibility given medical histories. Age was particularly crucial for determining medical information because children in this age range rarely have other inflammatory illnesses that would be of relevance, like autoimmune disorders. ASD children who had asthmatic generated significantly greater amounts of IL-17 in response to stimulating (121.3 pg/ml, 24.43 pg/ml to 839.4 pg/ml) comparing to ADHD individuals without asthma (62.73 pg/ml, 0.199 pg/ml to 4,750 pg/ml, p=0.020) and ADHD individuals with asthma (28.06 pg/ml, 10.14) (Figure 3-A). Similar to IL-17, ASD people with asthma produced significantly more IL-13 after PHA activation (1,011 pg/ml, 223.2 pg/ml to 9,105 pg/ml) than ADHD individuals with asthmatic (348.2 pg/ml, 38.12 pg/ml to 1,079 pg/ml; p=0.036) (Figure 3-B). When opposed to ADHD patients with asthma (9.74 pg/ml, 0.861 pg/ml to 18.13 pg/ml; p=0.011), ADHD individuals lacking observed asthma resulted in significantly more IL-4 (41.63 pg/ml, 0.162 pg/ml to 1,066 pg/ml) (Figure 3-C). Last but not least, there wasn't any discernible differentiation in IL-4 release between ADHD and ASD children (with or without reported asthma) (Figure 3-C).

Cytokine production in individuals with food allergies

Skin (except eczema) and pharmaceutical allergic were two other categories of individuals with allergy sub-categories that were too small to be subjected to independent data analysis (Table 4). There was no clear differentiation in IL-17, IL-13, or IL-4 secretion across diagnostic categories for any types of documented allergies, particularly eczema, or for environmental food intolerances.

Variations in the quantities of cytokine productions after activation for individuals with food allergies were noted. Food intolerance, IL-17, IL-13, and IL-4 levels were not significantly different between diagnostic categories for ASD, but there were changes between diagnostic categories (Figure 4-A-C). In comparison to ADHD individuals without documented allergies (51.19 pg/ml, 0.199 pg/ml to 4,759 pg/ml; p=0.022; p=0.011, correspondingly), ASD patients without documented allergies (87.36 pg/ml, 8.797 pg/ml to 1,789 pg/ml) and ADHD individuals with observed food intolerances (215.7 pg/ml, 25.24 pg/ml to 1172 pg/ml) (Figure 4-A). Furthermore, the ADHD children who had a dairy allergy had the highest total IL-17 output (Figure 4-A). In terms of IL-13, persons with ADHD (1.061 pg/ml, 0.029 pg/ml to 20.004 pg/ml, p=0.024) and ASD (1.114 pg/ml, 18.61 pg/ml to 11.105 pg/ml, p=0.029) who had not previously been diagnosed with a food allergy produced considerably higher quantities of IL-13 (3.12 pg/m, 317.2 pg/ml to 11.599 pg/ml) (Figure 4-B). Regarding IL-4, there was no noticeable difference between the study groups containing and without food intolerances (Figure 4-C).

Table [1]: General characteristics of the study participants

		Autism Spectrum Disorder (ASD) cases [n=55]	Attention-deficit/hyperactivity disorder (ADHD) cases [n=95]	Total patients [n=150]
Age at blood draw (years)		6.98	6.96	2.98
Gender	Males	47 (85.5%)	78 (82.11%)	97 (85%)
	Females	8 (14.5%)	17 (17.9%)	17 (15.0%)

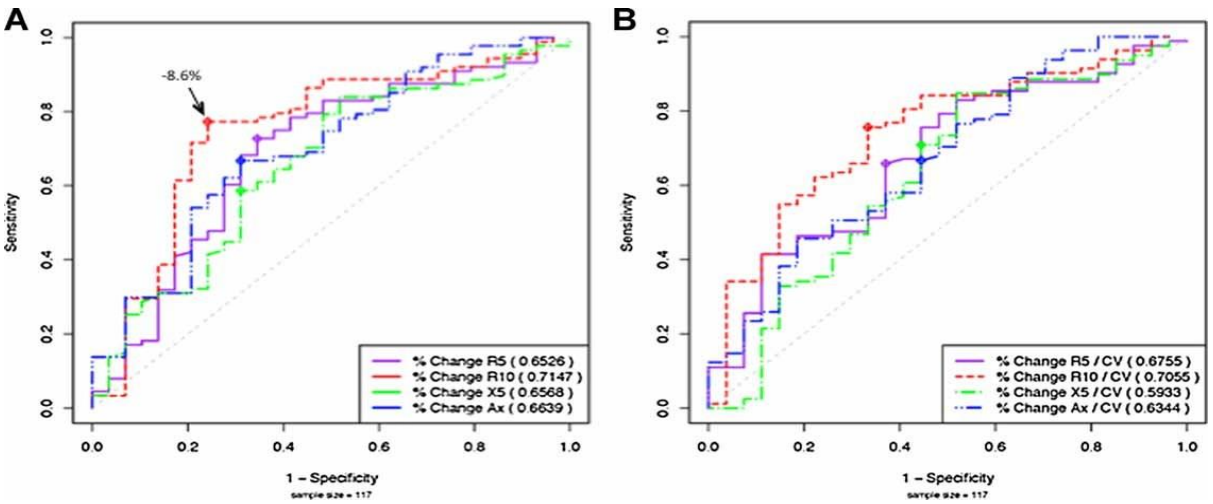

Figure [1]: Receiver operator characteristics for IOS are shown, contrasting asthma sufferers and non-asthmatics for R5, R10, X5, and AX. To determine the profiles of specificity and sensitivity for every testing variable, area-under-curve (AUC) is determined. For R10, the optimum cut-off level is provided. B: Bronchodilator reaction divided by CV, or ROC for CV index positively reaction indicated by CV index of ≥1

Table [2]: ROC Cut-Off Points

Measurement	1 − Specificity	Sensitivity	Cut-offs
Ax.CV	0.44	0.67	−0.66
X5.CV	0.44	0.71	−0.21
R20.CV	0.33	0.63	−0.32
R10.CV	0.33	0.76	−0.53
R5.CV	0.37	0.66	−0.59
AX	0.31	0.67	−29.11
X5	0.31	0.59	−18.15
R20	0.35	0.62	−5.91
R10	0.24	0.77	−8.58
R5	0.34	0.73	−11.24
FEV_1	0.40	0.54	6.23

Table [3]: Immune-mediated illnesses among study participants

	Autism Spectrum Disorder (ASD) cases [n=55]	Attention-deficit/hyperactivity disorder (ADHD) cases [n=95]	p-value
Parameters	No. (%)	No. (%)	
Asthma	15 (27.3)	7 (7.4)	0.025
Any allergy*	20 (36.4)	36 (38)	1.000
Skin allergy (excl. eczema)	1 (1.8)	2 (2.1)	1.000
Environmental (hay fever/seasonal allergies)	17 (30.9)	30 (31.6)	0.756
Eczema	19 (34.5)	33 (34.7)	1.000
Food allergy	5 (9.1)	11 (11.6)	0.874
Medication allergy	3 (5.5)	4 (4.21)	1.000
Any immune-mediated condition	35 (63.6)	53 (55.8)	0.868

Fisher's Exact Testing with two-tailed results was used to get P-values. * Environmental, food, pharmaceutical, and skin allergies are all included in the term "allergy"; eczema was researched independently.

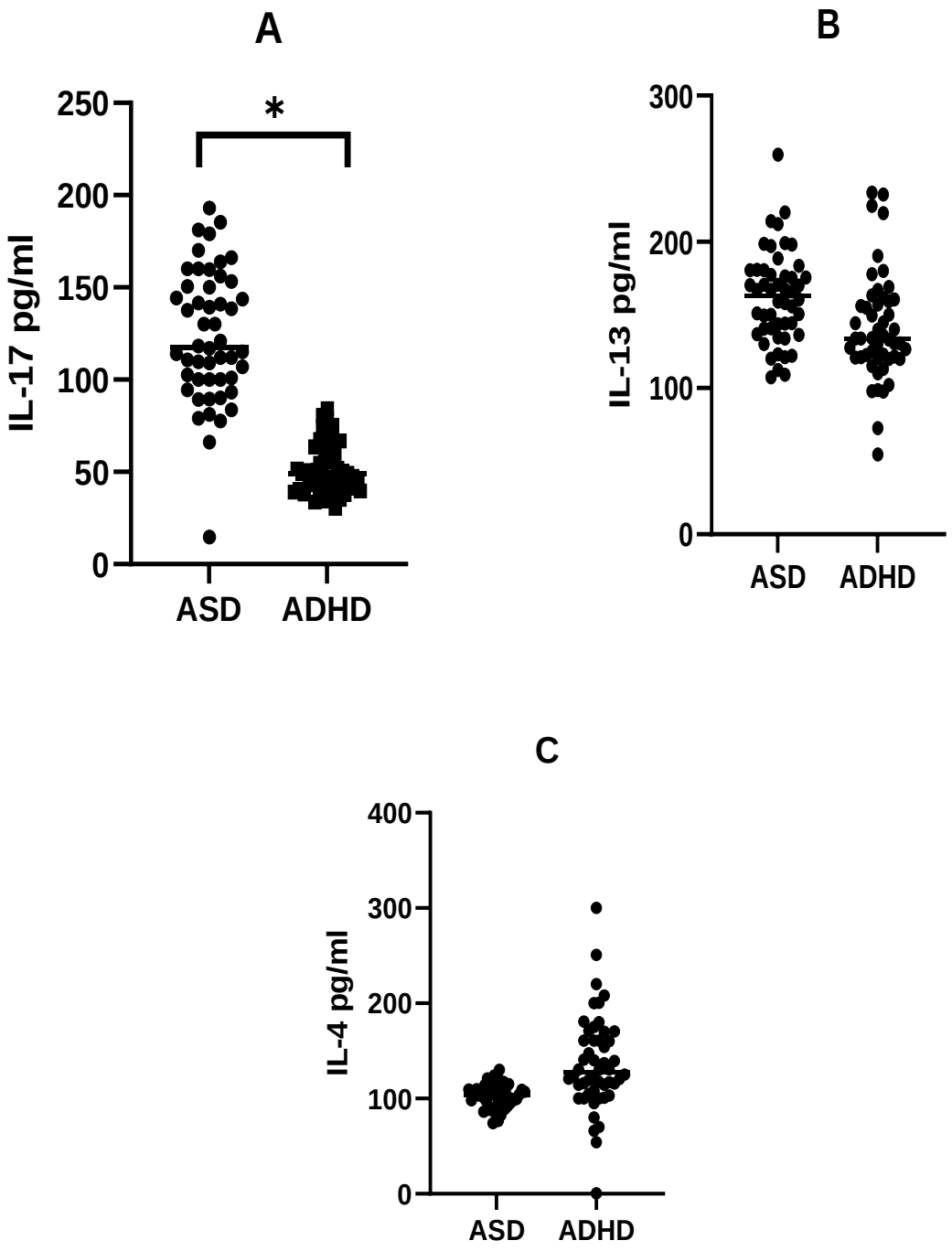

Figure [2]: IL-17, IL-13, and IL-4 cytokine production in PHA-stimulation cultured cells supernatants from ASD and ADHD subjects. A: PHA-stimulated IL-17 concentrations are significantly different in ASD patients in comparison to ADHD individuals (p=0.036). B: Within cohorts, there was no clear differentiation in the quantities of IL-13 that PHA induced in cell culture supernatants. C: PHA-stimulated IL-4 concentrations in cultured cells supernatants did not differ significantly across groups. P-values were calculated using a Mann-Whitney U t-test with two tails. (*p<0.05, **p<0.001). On a log10 Y-axis, bars show the median and interquartile range.

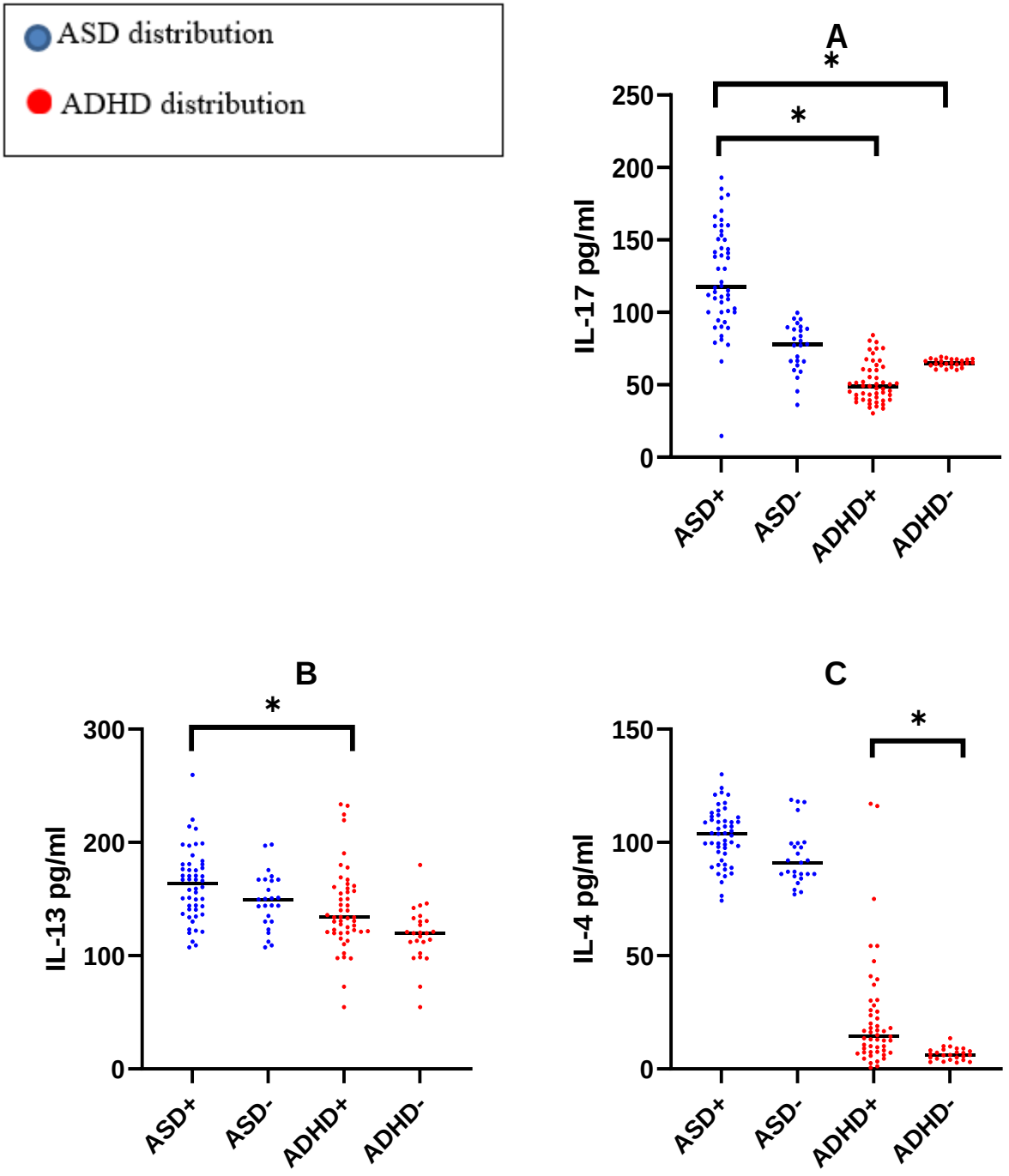

Figure [3]: Production of IL-17, IL-13, and IL-4 in individuals both with and without asthma who had ASD and ADHD A) PHA-stimulated IL-17 concentrations from ASD subjects with asthma vary markedly from ADHD participants with asthma (p=0.0192) and without asthma (p=0.020), respectively. B) PHA-stimulating IL-13 concentrations are significantly different in ASD individuals with asthma comparing to ADHD patients with asthma (p=0.036). C) Comparing ADHD patients with and without asthma, there was a substantial disparity in PHA-stimulated IL-4 concentrations, with the latter group of typically developing children producing more IL-4 (p=0.011, correspondingly). P-values were calculated using a Mann-Whitney U analysis with two tailed. (*p<0.05, **p<0.001). (-) asthma; (+) with asthma. On a $\log_{10}$ Y-axis, bars show the median and the interquartile ranges.

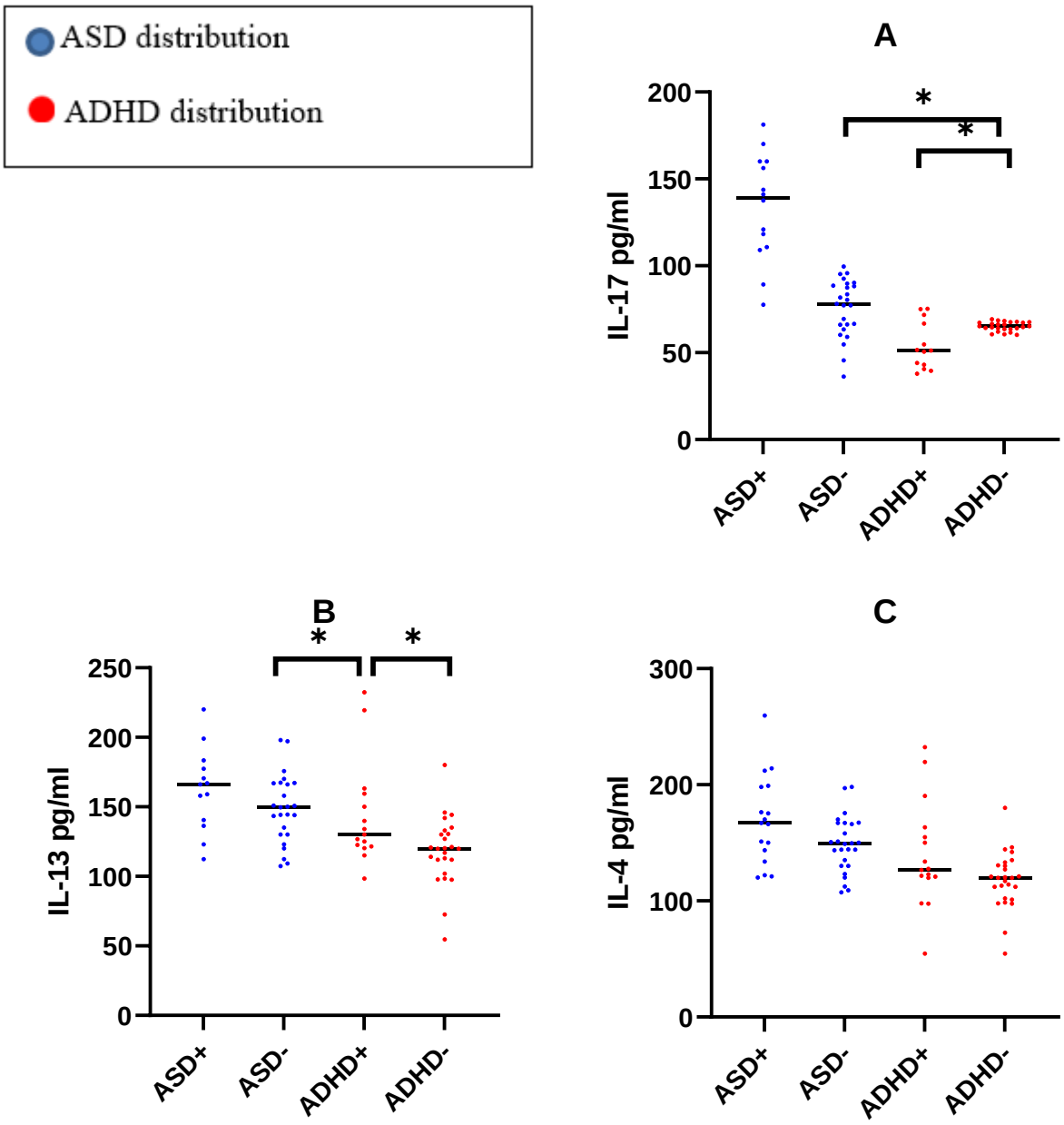

Figure [4]: Production of IL-17, IL-13, and IL-4 in individuals with and without documented food intolerances in ASD and ADHD. A: PHA-stimulated IL-17 concentrations in ASD subjects without known food allergies differed significantly from those in ADHD patients without known food allergies (p=0.022). Additionally, ADHD subjects with and without food intolerances had significantly different PHA-stimulated IL-17 concentrations (p=0.022). B: PHA-stimulated IL-13 concentrations from ADHD individuals with a documented food allergy were significantly higher than those from ASD patients without a food allergy (p=0.029) and ADHD patients without a documented allergy (p=0.024). C) PHA-stimulated IL-4 concentrations in cells cultured supernatants did not vary significantly across groups. P-values were estimated using a Mann-Whitney U test with two tails. (*p<0.05, **p<0.001). (+) having a food intolerance that has been recorded; (-) without a food allergy at all. On a log10 Y-axis, bars show the median and interquartile ranges.

DISCUSSION

In this work, the cellular release of the Th17 cytokines, IL-17, in addition to the Th2 cytokines, IL-13, and IL-4 were assessed, all of which are known to be connected with allergic immune-mediated diseases, in peripheral blood immune cells from ASD youngsters and ADHD cells from children with ASD produced more IL-17 after ex vivo PHA activation than ADHD subjects. Now it is widely known that people with ASD have abnormal immunological profiles [43] that are associated with behavioural profile deficiencies [44, 45]. The present research also shows that certain cases with ASD exhibit immunological hyperactivity, which may be

indicative of underlying inflammatory problems in these children. This is supported by the increasing yield of IL-17 in these children. These results may indicate chronic immune dysfunctions that could result in other immune-mediated disorders later in life, even if this immunological profile were likely far from pathogenic at the time of blood sample at 2–5 years of age. To answer this, extensive studies would be required.

Data from previous research on IL-17 release in ASD patients was contradictory. ASD children were evaluated in an earlier work by **Onore, *et al.*,** who looked at IL-17 release after PHA stimulating. They observed no significant changes in IL-17 release [46]. Additional research by **Enstrom *et al.*** measured the amounts of IL-23 and IL-17 in plasma from children. The researchers found that children with ASD had lower plasma concentration of IL-23 comparing to normal controls, but no statistically meaningful variations in IL-17 concentrations [47].

In the present study, ASD children had a reported incidence of asthma that was noticeably higher than that of ADHD group. This differed slightly from previously published results from a greater sample of children in the trial with ASD, where no appreciable variation was seen between children with ASD and children without ASD for parent-reported asthma [48, 49]. It is feasible that the research populations studied by **Onore, *et al.*** [46] and **Enstrom, *et al.*** [47] enclosed a smaller percentage of ASD participants with asthma, which may have lowered the magnitude of the variations noted in IL-17 concentrations between ASD patients and non ASD children. This is relevant to the contrast of the distinctions in IL-17 secretion after PHA-stimulating with the other previous papers.

In a study by **Al-Ayahi and Mostafa**, higher serum values of IL-17 were found in ASD and ADHD individuals compared to controls. These results are consistent with those reported in the current report, despite the fact that the researchers focused on serum concentrations as opposed to activated cell supernatants [50]. The demographics examined in the latest analysis and other research projects by the **Al-Ayadhi** group were improved for people with autoimmune disorders, the majority of whom showed autoantibodies to various antigens [51].

In the current study, ASD subjects with and without asthma and food intolerances were compared to determine the correlation between autism severity ratings and PHA-stimulated IL-17, IL-13, and IL-4 release. For ASD individuals with or without asthma and food intolerances, no relationship between autistic intensity ratings and cytokines secretion was seen. These findings imply that the intensity of autistic symptoms in ASD children with asthma and/or food allergies is not correlated with increased release of PHA-stimulated IL-17, IL-13, and IL-4.

IL-4 and IL-13 are both important cytokines in allergic asthma. In additional to IL-17, we saw a correlation among IL-13 and asthma in children with ASD. Asthmatic sufferers' predominant CD4+ cells generate both IL-4 and IL-13, which increases B-cell synthesis of IgE. Consequently, eosinophil recruiting, enhanced overexpression of cell adhesion molecules, and hypersecretion of mucous follow [52]. As a result, it was expected that participants with asthma might produce more IL-4 and IL-13, and we did find a link between IL-13 and ASD diagnoses, but not IL-4. Nevertheless, it was found that ADHD children without asthma had greater concentrations of IL-4 than ADHD children who had asthma. Contrary to popular belief, it was only assessed the IL-4 and IL-13 release from separated PBMC after PHA stimulating.

IL-17, IL-13, and IL-4 cytokines concentrations were also assessed in subjects with known allergy disorders. In the present study, the term "allergies" was used broadly and encompassed a number of subclasses of skin allergies in addition to environmental allergies, drug allergies, food allergies, or other allergies that were recorded. Allergy symptoms are a hypersensitive condition of the immune response. Only in the realm of food intolerances did we discover substantial variations across diagnostic categories in the current study. Between the diagnostic groups, there were no appreciable variations in the frequency of any additional allergy-related sub-categories. Earlier studies have found a strong link among ASD and a family medical history of allergies [49, 53, 54]. Others did not, nevertheless [55]. As a result, there are still significant inconsistencies in the link among allergy and ASD. Due to the current study's small sample size, it was not entirely representative of all pediatric patients.

In the current investigation, we assessed the occurrence of allergies and asthma amongst children included using clinician- and parent-reported responses to questionnaires given by qualified individuals. Despite the fact that research criteria were somewhat open-ended, the results point to the possibility that high IL-17 is related to immunological dysregulation brought on by atopy, especially in ASD children. It is not yet known how closely the observed neurological alterations in autism correspond to the higher production of PHA-stimulated IL-17 in early children with ASD.

IL-17 is generated in the central nervous system (CNS) under some pathological circumstances by a variety of periphery immune cells that have infiltrated the CNS, and to a lesser degree by astrocytes and microglia that are localized in the CNS. The most extensive amount of research has focused on IL-17's effect in multiple sclerosis (MS), while it has also been connected to a number of CNS illnesses, such as depression and ischemic brain injuries [56]. Observations have shown that inflammatory in the peripherals may affect brain activity and result in pathological alterations in the CNS because cells of the CNS have functioning IL-17 receptors [56]. IL-17 has the ability to directly cause neuronal injury in the parenchyma of the CNS whether by itself or in conjunction with other stimuli. Additionally, IL-17 signaling via receptors on microglia and astrocytes promotes the production of a wide range of pro-inflammatory cytokines as well as elements that support tissue regeneration and the resolution of inflammation. So, persistent inflammation and increased IL-17 generation in youngsters with co-morbid asthma and autism may eventually affect alterations and inflammation in the CNS.

In a study done by **Akdis et al.** [57], it was stated that the growth in allergy, autoimmune, and other chronic illnesses is caused by an increase in substances that damage the epithelial barrier, which is linked to industrialization, urbanisation, and modern living [58]. Additionally, it examines how the development of these disorders may be influenced by the immune responses to dysbiotic bacteria that pass through the broken barrier [59, 60].

In conclusion, it was shown that, depending on the neurodevelopmental outcomes, children who have asthma and/or food allergy have a varied immunological response. The current findings strongly suggest that increased IL-17 generation is associated with a co-morbidity that is frequently reported to be related to ASD and that it is also highly expressed in ASD children with asthma comparing to ADHD counterparts with the same stated problem. In addition, even though the medical phenotypes of ASD patients and typically developing controls overlaps, the underlying cellular role pertinent to that phenotype is significantly different, as evidenced by the comparison of the T cell reaction to mitogen stimulation between those children and controls. Additionally, there was an emphasis on the significance of considering co-morbid diseases when assessing the biological disparities between those with ASD and ADHD. Lastly, this study advances knowledge of the association between immune malfunction in children with two neuro-developmental condition and children who are typically developing, in addition to how these variations can affect the behaviours and co-morbidities connected to such diseases.

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