

## Salivary IgA Correlate with Recurrent Respiratory Infections and Hyperreactivity in Children with Autism

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### ABSTRACT

**AIM:** Autism spectrum disorders (ASD) are characterized by impairment in social interactions, communication deficits, and restricted interests and behaviors. Accumulating evidence suggests that dysregulation of the immune system may be involved in the pathophysiology of ASD. The aim of the study was to assess if the severity of clinical and behavioral parameters of autistic children was associated with low levels of secretory IgA (sIgA) in saliva. We hypothesized that a decreased immune response in children with autism would decrease the levels of sIgA, as is the predominant antibody isotype in saliva and a marker of mucosal immunity.

**MATERIALS AND METHODS:** Saliva samples were obtained from 3-10 year-old children with ASD and age-matched typically

developing Caucasian children from Patagonia region, Argentina.

**RESULTS AND CONCLUSIONS:** Autistic children with reduced levels of salivary IgA had a higher incidence of upper respiratory diseases compared to the controls. The reduction in sIgA levels also correlated inversely with the severity of the behavioral disorders. The patients with the most severe impairment in autism-related behaviors had the lowest levels of sIgA in the cohort studied. These findings suggest that sIgA could be an early indicator and possibly a biomarker of the dysregulation of the immune system in some children with autism. The characterization of immunological parameters in ASD has important implications for detection of a subset of individuals with ASD, and should be considered when designing therapeutic strategies to treat core symptoms and behavioral impairments of ASD.

**Key words:** Autism; sIgA; Saliva; Respiratory infections; Sensory hyper reactivity; Irritability

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### INTRODUCTION

Autism spectrum disorders (ASD) are characterized by significant impairment in reciprocal social interaction, as well as a markedly restricted, repetitive, and stereotyped behaviors in the early developmental period (American Psychiatric Association. Diagnostic and statistical manual of mental disorders 5th edn, Washington DC, 2013). In addition to behavioral symptoms, co-morbid clinical conditions are more common in children with autism compared to typically developing counterparts<sup>[1,2]</sup>. Although there is some

evidence suggesting that a subset of children with ASD may have a high incidence of respiratory infections, the data is not confirmed and based mostly on non-confirmed clinical evidence of respiratory infections unusually long in autistic. These observations led to the hypothesis that the immune system of a subgroup of ASD population that suffers from airways infections significantly more frequently than healthy control children during the first 3 years of life, is defective. A recent study also demonstrated that children with ASD are more likely to have middle ear infections and otitis-related complications than healthy counterparts<sup>[3]</sup>.

A dysregulated or abnormal immune response in children with ASD has been considered to play a potential and yet undetermined role in the etiology of autism<sup>[4,5]</sup>. Immune system abnormalities in children with autism include both enhanced autoimmunity and reduced immune function<sup>[6]</sup>. One of the most reported immune abnormalities in autistic children include an imbalance of serum immunoglobulin (Ig)<sup>[7]</sup> and cytokines levels<sup>[8]</sup>. A dysregulated immune system in children with autism could eventually lead to a higher risk of suffering infections at early ages compared to the non-autistic population.

Immunoglobulins are part of the humoral immune response, the net result of a specific response orchestrated by the complex interaction between dendritic cells, T cells, and Ig-producing B cells. Ig levels are therefore used to measure immune development and humoral immune function. IgA is the most abundant immunoglobulin in the human body, and performs a very specialized role, which involves mucosal immunity and protection against infection<sup>[9]</sup>. IgA constitutes the predominant immunoglobulin isotype in secretions, including saliva and it is the first line of defense of the host against pathogens, which colonize or invade surfaces bathed by external secretions<sup>[10]</sup>. Secretory IgA is the key immunoglobulin in respiratory and gastrointestinal tracts, which is the actual interface between the environment and the human body<sup>[11]</sup>. Low levels of IgA in saliva have been reported as a risk factor for upper respiratory infection<sup>[12]</sup>. Moreover, recurrent sinopulmonary infections of the respiratory system are the most common findings in individuals with IgA deficiency<sup>[13-15]</sup>. In a prospective Swedish study in children, IgA-deficient patients were found to have an increased risk of pseudocroup at the first year of life<sup>[16]</sup>.

The primary aim of this study was to assess sIgA in saliva of children with autism and age-matched healthy controls and the frequency of respiratory tract infections diagnosed in the first 3 years of life in children with autism in comparison to controls. In addition, associations between salivary levels of sIgA and the severity of clinical behavioral outcomes related to autism were examined.

## MATERIALS AND METHODS

### Ethics statement and subjects

Parents of all patients and controls gave informed consent to participate in this study. The Medical Ethics Committee of the local Hospital in Bahia Blanca city assessed the study and all study procedures were conducted in accordance with the Declaration of Helsinki (1964). All participants were from lower middle class and received medical attention from public national healthcare systems. The ASD group was comprised of 35 male Caucasian children from Patagonia region (Argentina) with a median age of 5.7 years (3-10 years). The typically developing children group was comprised of 35 male Caucasian children also from Patagonia region, with a median age of 5.9 years (3-10 years).

The exclusion criteria for all participants included prematurity, low birth weight, Apgar score < 7, and presence of other medical, genetic,

metabolic, or other concurrent physical, mental, or neurological disorder. Exclusion criteria for the control group were if they had any first- or second-degree relatives diagnosed with ASD.

### Behavioral assessment

The diagnosis of autism was based on DMS-V criteria<sup>[17]</sup>. Diagnoses were confirmed through clinical examinations using the Autism Diagnostic Observation Schedule (ADOS). The ADOS is a semi-structured and standardized assessment in which the researcher observes the social interaction, communication, play, and imaginative use of materials for children suspected of having autism or ASD. Sensory profiles were evaluated using the Short Sensory Profile (SSP)<sup>[18]</sup>, which is a standardized parent questionnaire that was developed as a screening instrument to identify children with sensory processing difficulties and associated behaviors. It is important to note that lower scores on this measure reflect greater impairment. Irritability was measured with The Aberrant Behavior Checklist (ABC)<sup>[19]</sup> which is a 58-item questionnaire designed to assess the presence and severity of disruptive behaviors domains (irritability, lethargy, stereotypy, hyperactivity, and inappropriate behavior). Medical histories and physical examinations were performed on all children. Medical histories for acute respiratory illnesses were collected from clinician's records.

### Determination of secretory IgA levels in saliva

Saliva is an easily accessible fluid useful for monitoring systemic health that can be obtained by noninvasive and non-stressful methods. In this study, we chose saliva because of the non-invasive approach that is particularly important for autistic children, who are very prone to stress. Mothers of autistic children were present during the procedure in order to avoid or minimize stress in those children. All samples of saliva were collected from all subjects, in fasting state, in the morning hours (between 9 am and 11 am). After rinsing their mouth with filtered water, saliva was collected by suction technique using 2 mL disposable syringe. Immediately samples were centrifuged (15 min at 10,000 rpm, 4°C) to remove cells and debris. Collected samples were frozen at -80°C and stored until analysis. Salivary IgA levels were determined by the ELISA (Salivary Secretory IgA, Enzyme Immunoassay Kit, Salimetrics, LLC, USA) following the manufacturer's instructions. All samples, standards and controls were run in duplicate.

### Statistical Analysis

The sIgA levels are expressed as mean  $\pm$  SD and range values with coefficient of variation (CV%). Since normality was not presumed in sIgA levels, a nonparametric method, the Mann-Whitney test was used to compare between the groups. Student's t-test was applied to compare variables of a normal distribution. Statistical analysis was performed using GraphPad Prism 6.0. For all the tests, a *p*-value of < 0.05 was considered statistically significant.

## RESULTS

Demographic characteristics of all children included in the study were summarized in Table 1. Overall, the groups of autistic children did not differ significantly from controls in demographics and birth-linked parameters.

The frequency of acute respiratory infections at early ages in children with autism in comparison to controls is shown in Table 2. Children having autism showed a significantly higher frequency of upper respiratory infections compared to normal peers in the first 3 years of life. For lower respiratory infections, autistic group was

**Table 1** Socio-demographic and clinical characteristics of the participants.

Characteristic	Controls ( <i>n</i> = 35)	ASD group ( <i>n</i> = 35)	<i>p</i>
Male - Female	35-0	35-0	
Age, mean (SD) yr	5.9 (2.7)	5.7 (2.9)	
Birth weight, mean(SD), g	3.380 (581.9)	3.250 (525.8)	NS
Delivery n(%)			NS
Natural	26 (74.2)	28 (80.0)	
Caesarean section	9 (25.7)	7 (20.0)	
Apgar score, mean(SD)	9.5 (0.2)	9.4 (0.3)	NS
Gestational age, mean(SD) wk	39.2 (2.1)	38.8 (6.0)	NS
Maternal age, mean(SD) yr	29.9 (5.5)	32.3 (6.0)	NS

NS: no significant differences between groups.

**Table 2** Frequency of acute respiratory infections in children during the 3 first years of life.

Clinical diagnosis	Health Controls ( <i>n</i> = 35)		ASD cases ( <i>n</i> = 35)	
	<i>n</i>	%	<i>n</i>	%
Upper respiratory infections				
pharyngitis	18	60	26	85*
pseudocroup	4	13	8	25*
Lower respiratory infections				
bronchiolitis	8	28	11	38*
pneumonia	7	24	9	29
bronchitis	2.7	9	3	9

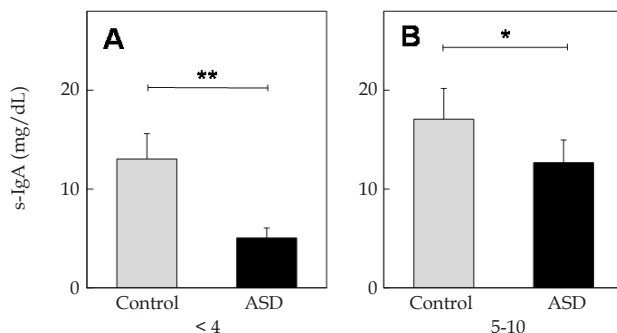
\* Statistically significant ( $p < 0.005$ ) between ASD children and healthy controls that participated in this study. Data was collected from clinical records.

found to have significantly increased rate of pneumonia compared to age-matched neurotypical children.

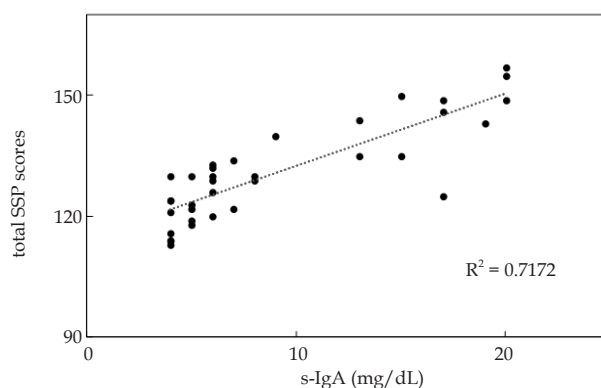
Figure 1 shows the analysis of levels of sIgA in saliva of autism patients and in age- and sex-matched healthy controls. We observed significant differences in the levels of sIgA present in the saliva of autistic patients regardless of age, compared to the values measured in the saliva of healthy individuals. Children with autism younger than 4 years had a significantly lower median level of s-IgA compared to neurotypical age-matched controls. The frequency of children younger than 4 years with salivary IgA levels  $< 7$  mg/dL was significantly higher in ASD group (83.0 %) as compared to healthy controls (17%;  $p < 0.05$ ). The frequency of ASD children with reduced levels of salivary sIgA decreased with age. Contrary to the high prevalence of younger ASD children with low levels of salivary sIgA, subjects with low salivary levels of IgA in the ASD group aged 4-10 showed a lower frequency (30%, Figure 1).

In addition to the evaluation of salivary IgA levels, we also examined the possible association of sIgA with clinical features associated with autism. All autism cohort exhibited atypical sensory processing patterns with definitive difference in the total SSP score. The SSP value for the whole ASD group was  $120 \pm 18$  reflecting great impairment. Although the general sensory processing was affected in all individuals of our study group, tactile sensitivity and auditory filtering were the most affected domains. Based on total SSP assessment scores in children with ASD, low levels of s-IgA in saliva correlated with severity of sensory processing ( $r = 0.81$ ,  $p < 0.005$ , Figure 2), being both auditory and tactile hyperreactivity the most prevalent sensory impairment symptoms in ASD children.

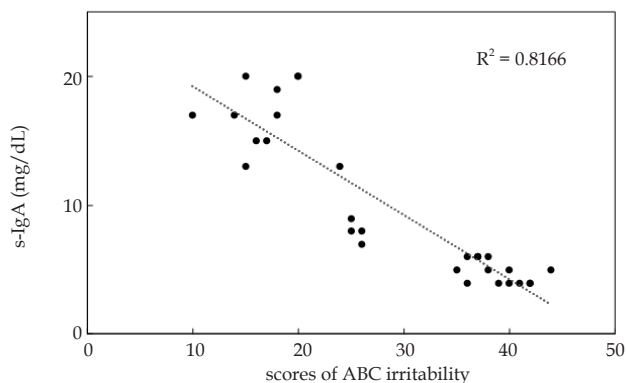
The possible association between levels of sIgA in saliva and aberrant behavior was assessed using the irritability ABC subscales scores (Figure 3). When the ASD subject population was analyzed



**Figure 1** Salivary s-IgA levels (mg/dL) in autism patients and healthy controls. A) s-IgA levels in autistic children (black bars) below 4 years were significantly lower compared to those of age-matched controls (grey bars) (\*\* $p < 0.001$ ,  $n = 18$ , mean age (SD): 3.2 (0.6)). B) s-IgA levels in autistic children above of 5 years were significantly lower to those of age-matched control,  $n = 17$ , mean age (SD): 8.2 (1.3) (\* $p < 0.05$ ).



**Figure 2** Correlation between levels of sIgA and total Short Sensory Profiles (SSP) scores in autistic children.



**Figure 3** Negative correlations between level of s-IgA and Aberrant Behavior Checklist (ABC) subscale scores in children with autism.

without the control subjects, sIgA levels correlated inversely with extreme irritability ( $r = -0.90$ ), thus the lower the salivary s-IgA level, the higher the ABC subscale scores, indicating increased severity of behavioral symptoms that are related with autism.

## DISCUSSION

Our current data suggest that children with autism younger than 4 years of age had a poorly developed IgA secretory system compared to typically developing age-matched controls indicating a suboptimal humoral function in the ASD population. The IgA secretion on respiratory mucosal surfaces, which is the very first step of immune

defense against mucosal pathogens, is significantly lower in ASD children, which is consistent with the fact that particularly in this subgroup of children there is an increased frequency of infections of the upper respiratory tract during the first years of life. It is well known that in children younger than 4 years there is transient and temporally IgA deficient due to a delayed ontogeny of IgA system<sup>[13]</sup>. According to our present results, it could be suspected that this process may be even slower in infants with autism compared to their healthy counterparts. Previous studies have reported changes in several aspects of the immune system in children with autism including altered levels of immunoglobulins as reviewed by Ashwood and coworkers<sup>[7]</sup>. Although several studies had been conducted on the systemic immune status of children with ASD, none of these had focused on the levels of IgA in saliva. Saliva has progressively become a preferred specimen choice for biological analysis for some groups of patients, because it is convenient, noninvasive and stress-free<sup>[20]</sup>. This is especially important for autistic children, who often have exaggerated stress reactions.

Recurrent infections of the upper respiratory system are the most common clinical findings and the most commonly treated acute problems in general pediatrics primary care in children with IgA deficiency<sup>[13,15]</sup>. According to our data, and in agreement with these previous evidences, respiratory diseases were diagnosed significantly more often among those children with autism than among controls, suggesting a strong association between low levels of sIgA and a high frequency of respiratory infections, at least at early ages. The higher prevalence of respiratory infections in autistic children younger than 4 years in comparison with the typical developmental age-matched population, could be, at least, partly explained by the high frequency of low salivary IgA levels in ASD group (83.0 % vs 17%). Based on clinician's reports on childhood infection history, the majority of infections occurring in non-autistic pediatric cohort were of viral origin and, therefore did not require antibiotics. By contrast, in ASD children antibiotics were frequently used to treat recurrent infections, especially affecting middle ear, upper airways and gut suggesting that bacteria were the cause of the infections or a complicating factor in viral respiratory diseases. In any case, infants with autism were more exposed to antibiotics at early ages than the non-autistic children.

Exposure to antibiotics, especially during critical developmental times might be detrimental especially by disrupting the functions of the microbiota. Disruption of the actively developing infant gut microbiota at early ages may disrupt metabolic and immune development and affect human health<sup>[21]</sup>. The use of oral broad-spectrum antibiotics can hamper or damage a healthy normal microflora, especially in vulnerable infants. Many infections that occur in the first 3 years of life, concomitant with early gut colonization, are treated with oral broad-spectrum antibiotics that can perturb the gut microbiota<sup>[22]</sup> leading to the risk of gut dysbiosis and subsequent GI disturbances, which are frequently described in the ASD population<sup>[23,24]</sup>.

Recognizing underlying GI conditions or other medical problems may be difficult in individuals with no or limited verbal capability. This is especially important for children with autism that have impaired communication skills. Since early-life exposure to antibiotics to treat frequent respiratory illness is a risk factor for GI disorders associated with dysbiosis, and these clinical manifestations are common features of IgA deficiencies, the analysis of sIgA levels in saliva could provide a potential non-invasive, easy to perform and non-stressful auxiliary screening tool to detect a subset of ASD children with immune dysregulation and potential risk to develop GI dysfunction. We strongly recommend considering potential GI

disturbances in all autistic children that showed repetitive episodes of airways infection illness in early life that concurred with reduced levels of salivary sIgA.

In autism there is evidence of altered immunity occurring both immediately after birth and throughout disease progression<sup>[25]</sup>. Immune function may also relate to social behavioral outcomes<sup>[25]</sup>. Numerous published findings have identified widespread differences in the immune systems of children with ASD, both at the systemic and cellular levels<sup>[6]</sup>. These differences have been associated with impairments in the core features of ASD as well as other aberrant behaviors, decreased adaptability and more impaired cognition in children with ASD<sup>[25,26]</sup>. For example, increased plasma levels of cytokines were observed in autistic children compared with age-matched controls, and this cytokine production was associated with more aberrant behaviors<sup>[27]</sup>. Moreover, significantly reduction in specific Ig levels correlated with behavioral severity in children with autism compared with age-matched typically developing children, suggesting an underlying defect in immune function<sup>[28]</sup>. In the present study, we showed that deficiencies in salivary sIgA levels were strongly associated with severe impairments in some clinical features in ASD group, strengthening the association between immune dysfunction and at least a subset of behaviors associated with autism. This association has been vastly explored by researchers who reported increased pro-inflammatory markers as the most consistent observation among the immunological findings in ASD<sup>[29,30]</sup>. As noted, many of these studies highlight a connection between immune dysregulation and more impaired behaviors. These findings suggest a pro-inflammatory immune profile prevalent within the ASD population, or at least in a subgroup of the population, where immune activation may be linked to more impaired behavioral symptomatology<sup>[31]</sup>. Given the heterogeneity of ASD, there remains a great need to further elucidate immune dysfunction in children with the disorder, and to work towards establishing immune endophenotypes within the broader spectrum in order to better target current and future therapies.

Although our present data is in agreement with current studies that emphasize that ASD children can be clustered into subgroups based on immune responses and then assessed for behavioral outcomes, the findings of this study needs to be carefully interpreted because of the small sample size and to the lack of other studies involving the estimation of salivary sIgA levels in children with autism.

## CONCLUSIONS

In the present study, autistic children with severe over sensory reaction, mostly tactile and auditory hyperreactivity, and extreme irritability showed reduced levels of salivary IgA. Although our present results are not conclusive about a partial IgA deficiency in the ASD cohort older than 4 years of age, it is possible to consider low level of sIgA in saliva as a promising, novel, non-stressfully and noninvasive screening instrument to help to identify a subset of ASD children with inappropriate immune responses. Given the high prevalence of GI disorders and autoimmunity in autism, that are also very common clinical features of IgA deficiencies, these results highlight the importance of further systematic immunological studies in children with autism. Further research involving larger-scale studies needs to be performed to confirm these findings and to help the identification of immune subphenotypes within the ASD population. Such information will be useful not only in the molecular diagnosis of ASD, but also in the development of targeted therapies to address the specific biological deficits manifested in clinically relevant subgroups of individuals with ASD.

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